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METHOPRENE

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CHAPTER I: HOW TO REGISTER UNDER A REGISTRATION STANDARD

A. Organization of the Standard
B. Purpose of the Standard
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D. "Product Specific" Data and "Generic" Data
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A. Organization of the Standard

This first chapter explains the purpose of a Registration Standard and summarizes the legal principles involved in registering or reregistering under a Standard. The second chapter sets forth the requirements that must be met to obtain or retain registration for products covered by this particular Registration Standard. In the remaining chapters, the Agency reviews the available data by scientific discipline, discusses the Agency's concerns with the identified potential hazards, and logically develops the conditions and requirements that would reduce those hazards to acceptable levels.

B. Purpose of the Standard

Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) provides that "no person in any State may distribute, sell, offer for sale, hold for sale, ship, deliver for shipment, or receive (and having so received) deliver or offer to deliver, to any person any pesticide which is not registered with the Administrator [of EPA]." To approve the registration of a pesticide, the Administrator must find, pursuant to Section 3 (c)(5) that:

> (A) its composition is such as to warrant the proposed claims for it;
>
> (B) its labeling and other material required to be submitted comply with the requirements of this Act;
>
> (C) it will perform its intended function without unreasonable adverse effects on the enviroinment; and
>
> (D) when used in accordance with widespread and commonly recognized practice it will not generally cause unreasonable adverse effects on the environment.

In making these findings, the Agency reviews a wide range of data which registrants are required to submit, and assesses the risks and benefits associated with the use of the proposed pesticide. But the established approach to making these findings has been found to be defective on two counts:

First, EPA and its predecessor agency, the United States Department of Agriculture (USDA), routinely reviewed registration applications on a "product by product" basis, evaluating each product-specific application somewhat independently. In the review of products containing similar components, there was little opportunity for a retrospective review of the full range of pertinent data available in Agency files and in the public literature. Thus the "product by product" approach was often inefficient and sometimes resulted in inconsistent or incomplete regulatory judgments.

Second, over the years, as a result of inevitable and continuing advances in scientific knowledge, methodology, and policy, the data base for many pesticides came to be considered inadequate by current scientific and regulatory standards. Given the long history of pesticide regulation in several agencies, it is even likely that materials may have been lost from the data files. When EPA issued new requirements for registration in 1975 (40 CFR 162) and proposed new guidelines for hazard testing in 1978 (43 FR 29686, July 10, 1978 and 43 FR 37336, August 2, 1978), many products that had already been registered for years were being sold and used without the same assurances of human and environmental safety as were being required for new products. Because of this inconsistency, Congress directed EPA to reregister all previously registered products, so as to bring their registrations and their data bases into compliance wtih current requirements, [See FIFRA Section 3(g)].

Facing the enormous job of re-reviewing and calling in new data for the approximately 35,000 current registrations, and realizing the inefficiencies of the "product by product" approach, the Agency decided that a new, more effective method of review was needed.

A new review procedure has been developed. Under it, EPA publishes documents called Registration Standards, each of which discusses a particular pesticide active ingredient. Each Registration Standard summarizes all the data available to the Agency on a particular active ingredient and its current uses, and sets forth the Agency's comprehensive position on the conditions and requirements for registration of all existing and future products which contain that active ingredient. These conditions and requirements, all of which must be met to obtain or retain full registration or reregistration under Section 3(c)(5) of FIFRA, include the submission of needed scientific data which the Agency does not now have, compliance with standards of toxicity, composition, and packaging, and satisfaction of the data compensation provisions of FIFRA Section 3(c)(1)(D).

The standard will also serve as a tool for product classification. As part of the registration of a pesticide product, EPA may classify each product for "general use" or "restricted use" [FIFRA Section 3(d)]. A pesticide is classified for "restricted use" when some special regulatory restriction is needed to ensure against unreasonable adverse effects to man or the environment. Many such risks of unreasonable adverse effects can be lessened if expressly-designed label precautions are strictly followed. Thus the special regulatory restriction for a "restricted use" pesticide is usually a requirement that it be applied only by, or under the supervision of, an applicator who has been certified by the State or Federal government as being competent to use the pesticide safely, responsibly, and in accordance with label directions. A restricted-use pesticide can have other regulatory restrictions [40 CFR 162.11(c)(5)] instead of, or in addition to, the certified applicator requirement. These other regulatory restrictions may include such actions as seasonal or regional limitation on use, or a requirement for the monitoring of residue levels after use. A pesticide classified for "general use," or not classified at all, is available for use by any individual who is in compliance with State or local regulations. The registration Standard review compares information about potential adverse effects of specific uses of the pesticide with risk criteria listed in 40 CFR 162.11(c), and thereby determines whether a product needs to be classified for "restricted use." If the Standard does classify a pesticide for "restricted use," this determination is stated in the second chapter.

C. Requirement to Reregister Under the Standard

FIFRA Section 3(g), as amended in 1978, directs EPA to reregister all currently registered products as expeditiously as possible. Congress also agreed that reregistration should be accomplished by the use of Registration Standards.

Each registrant of a currently registered product to which this Standard applies, and who wishes to continue to sell or distribute his product in commerce, must apply for reregistration. That application must contain proposed labeling that complies with this Standard.

EPA will issue a notice of intent to cancel the registration of any currently registered product to which this Standard applies if the registrant fails to comply with the procedures for reregistration set forth in the Guidance Package which accompanies this Standard.

D. "Product-Specific" Data and "Generic" Data

In the course of developing this Standard, EPA has determined the types of data needed for evaluation of the properties and effects of products to which the Standard applies, in the disciplinary areas of Product Chemistry, Environmental Fate, Toxicology, Residue Chemistry, and Ecological Effects. These determinations are based primarily on the data Guidelines proposed in 43 FR 29696, July 10, 1978; 43 FR 37336, August 22, 1978; and 45 FR 72948, November 3, 1980, as applied to the use patterns of the products to which this Standard applies. Where it appeared that data from a normally applicable Guidelines requirement were actually unnecessary to evaluate these products, the Standard indicates that the requirement has been waived. On the other hand, in some cases studies not required by the Guidelines may be needed because of the particular composition or use pattern of products the Standard covers; if so, the Standard explains the Agency's reasoning. Data guidelines have not yet been proposed for the Residue Chemistry discipline, but the requirements for such data have been in effect for some time and are, the Agency believes, relatively familiar to registrants. Data which we have found are needed to evaluate the registrability of some products covered by the Standard may not be needed for the evaluation of other products, depending upon the composition, formulation type, and intended uses of the product in question. The Standard states which data requirements apply to which product categories. (See the third chapter.) The various kinds of data normally required for registration of a pesticide product can be divided into two basic groups:

1. Data that are product-specific, i.e., data that relate only to the properties or effects of a product with a particular composition (or a group of products with closely similar composition); and

2. Generic data that pertain to the properties or effects of a particular ingredient, and thus are relevant to an evaluation of the risks and benefits of all products containing that ingredient (or all such products having a certain use pattern), regardless of any such product's unique composition.

The Agency requires certain "product-specific" data for each product to characterize the product's particular composition and physical/chemical properties (Product Chemistry), and to characterize the product's acute toxicity (which is a function of its total composition). The applicant for registration or reregistration of any product, whether, it is a manufacturing-use or end-use product, and without regard to its intended use pattern, must submit or cite enough of these kinds of data to allow EPA to evaluate the product. For such purposes, "product-specific" data on any product other than the applicant's are irrelevant, unless the other product is closely similar in composition to the applicant's. (Where it has been found practicable to group similar products for purposes of evaluating, with a single set of tests, all products in the group, the Standard so indicates.) "Product-specific" data on the efficacy of particular end-use products are also required where the exact formulation may affect efficacy and where failure of efficacy could cause public health problems.

All other data needed to evaluate pesticide products concern the properties or effects of a particular ingredient of products (normally a pesticidally active ingredient, but in some cases a pesticidally inactive, or "inert," ingredient). Some data in this "generic" category are required to evaluate the properties and effects of all products containing that ingredient [e.g., the acute LD-50 of the active ingredient in its technical or purer grade; see proposed 40 CFR 163.81-1(a), 43 FR 37355].

Other "generic" data are required to evaluate all products which both contain a particular ingredient and are intended for certain uses (see proposed 40 CFR 163.82-1, 43 FR 37363, which requires subchronic oral testing of the active ingredient with respect to certain use patterns only). Where a particular data requirement is use-pattern dependent, it will apply to each end-use product which is to be labeled for that use pattern (except where such end-use product is formulated from a registered manufacturing-use product permitting such formulations) and to each manufacturing-use product with labeling that allows it to be used to make end-use products with that use pattern. Thus, for example, a subchronic oral dosing study is needed to evaluate the safety of any manufacturing-use product that legally could be used to make an end-use, food crop pesticide. But if an end-use product's label specified it was for use only in ways that involved no food/feed exposure and no repeated human exposure, the subchronic oral dosing study would not be required to evaluate the product's safety; and if a manufacturing-use product's label states that the product is for use only in making end-use products not involving food/feed use or repeated human exposure, that subchronic oral study would not be relevant to the evaluation of the manufacturing-use product either.

If a registrant of a currently registered manufacturing-use or end-use product wishes to avoid the costs of data compensation (under FIFRA Section 3(c)(1)(D) or data generation [under Section 3(c)(2)(B)] for "generic" data that are required only with respect to some use patterns, the registrant may elect to delete those use patterns from his labeling at the time he reregisters his product. An applicant for registration for of a new product under this Standard may similarly request approval for only certain use patterns.

E. Data Communications Requirements under FIFRA 3(c)(1)(D)

Under FIFRA Section 3(c)(1)(D), an applicant for registration, reregistration, or amended registration must offer to pay compensation for certain existing data the Agency has used in developing the Registration Standard. The data for which compensation must be offered are all data which are described by all the following criteria:

1. The data were first submitted to EPA (or to its predecessor agencies, USDA OR FDA), on or after January 1, 1970.
2. The data were submitted to EPA (or USDA OR FDA) by some other applicant or registant in support of an application for an experimental use permit, an amendment adding a new use to a registration, or for registration, or to support or maintain in effect an existing registration;
3. They are the kind of data which are relevant to the Agency's decision to register or reregister the applicant's product under the Registration Standard, taking into account the applicant's product's composition and intended use pattern(s);
4. The Agency has found the data to be valid and usable in reaching regulatory conclusions; and
5. They are not data for which the applicant has been exempted by FIFRA Section 3(c)(2)(D) from the duty to offer to pay compensation. (This exemption applies to the "generic" data concerning the safety of an active ingredient of the applicant's product, not to "product-specific" data. The exemption is available only to applicants whose product is labeled for end-uses for which the active ingredient in question is present in the applicant's product because of the use of another registered product containing that active ingredient which is purchased from another producer.)

An applicant for reregistration of an already registered product under this Standard, or for registration of a new product under this Standard, accordingly must determine which of the data used by EPA in developing the Standard must be the subject of an offer to pay compensation, and must submit with the application the appropriate statements evidencing compliance with FIFRA Section 3(c)(1)(D).

An applicant would never be required to offer to pay for "product specific" data submitted by another firm. In many, if not in most cases, data which are specific to another firm's product will not suffice to allow EPA to evaluate the applicant's product, that is, will not be useful to the Agency in determining whether the applicant's product is registrable. There may be cases, however, where because of close similarities between the composition of two or more products, another firm's data may suffice to allow EPA to evaluate some or all of the "product-specific" aspects of the applicant's product. In such a case, the applicant may choose to cite data instead of submitting these data from tests on his own product, and if he chooses that option, he would have to comply with the offer-to-pay requirements of Section 3(1)(D) for those data.

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Each applicant for registration or reregistration of a manufacturing-use product, and each applicant for registration or reregistration of an end-use product, who is not exempted by FIFRA Section 3(c)(2)(D), must comply with the Section 3(c)(1)(D) requirements with respect to each item of "generic" data that relates to that product's intended uses.

A detailed description of the procedures an applicant must follow in applying for reregistration (or new registration) under this Standard is found in the Guidance Package for this Standard.

F. Obtaining Data to Fill "Data Gaps"; FIFRA 3(c)(2)(B)

Some of the kinds of data EPA needs for its evaluation of the properties and effects of products to which this Standard applies have never been submitted to the Agency (or, if submitted, have been found to have deficiencies rendering them inadequate for making registrability decisions) and have not been located in the published literature search that EPA conducted as part of preparing this Standard. Such instances of missing but required data are referred to in the Standard as "data gaps."

FIFRA Section 3(c)(2)(B), added to FIFRA by the Congress in 1978, authorizes EPA to require registrants to which a data requirement applies to generate (or otherwise produce) data to fill such "gaps" and submit those data to EPA. EPA must allow a reasonably sufficient period for this to be accomplished. If a registrant fails to take appropriate and timely steps to fill the data gaps identified by a section 3(c)(2)(B) order, the product's registration may be suspended until the data are submitted. A mechanism is provided whereby two or more registrants may agree to share in the costs of producing data for which they are responsible.

The Standard lists, in the third chapter, the "generic" data gaps and notes the classes of products to which these data gaps pertain. The Standard also points out that, to be registrable under the Standard, a product must be supported by certain required "product-specific" data. In some cases, the Agency may possess sufficient "product-specific" data on one currently registered product, but may lack such data on another. Only those Standards which apply to a very small number of currently registered products will attempt to state definitively the "product-specific" data gaps on a "product by product" basis. (Although the Standard will in some cases note which data that EPA does possess would suffice to satisfy certain "product-specific" data requirements for a category of products with closely similar composition characteristics.)

As part of the process of reregistering currently registered products, EPA will issue Section 3(c)(2)(B) directives requiring the registrants to take appropriate steps to fill all identified data gaps -- whether the data in question are "product-specific" or "generic" -- in accordance with a schedule.

Persons who wish to obtain registrations for new products under this Standard will be required to submit (or cite) sufficient "product-specific" data before their applications are approved. Upon registration, they will be required under Section 3(c)(2)(B) to take appropriate steps to submit data needed to fill "generic" data gaps. (We expect they will respond to this reqirement by entering into cost-sharing agreements with other registrants who previously have been told they must furnish the data.) The Guidance Package for this Standard details the steps that must be taken by registrants to comply with Section 3(c)(2)(B).

Registrants are reminded that Section 6(a)(2) of FIFRA requires you at any time to submit factual information raising concerns of possible unreasonable adverse effects of a pesticide. You should notify the Agency of interim results of studies in progress if those results show possible adverse effects.

G. Amendments to the Standard

Applications for registration which propose uses or formulation that are not presently covered by the Standard, or which present product compositions, product chemistry data, hazard data, toxicity levels, or labeling that do not meet the requirements of the Standard, will automatically be considered by the Agency to be requests for amendments to the Standard. In response to such applications, the Agency may request additional data to support the proposed amendment to the Standard, or may deny the application for registration on the grounds that the proposed product would cause unreasonalbe adverse effects to the environment. In the former case, when additional data have been satisfactorily supplied, and providing that the data do not indicate the potential for unreasonable adverse effects, the Agency will then amend the Standard to cover the new registration.

Each Registration Standard is based upon all the data and information available to the Agency's reviewers on a particular date prior to the publication date. This "cut-off" date is stated at the beginning of the second chapter. Any subsequent data submissions and any approved amendments will be incorporated into the Registration Standard by means of addenda, which are available for inspection at EPA in Wahsington, D.C., or copies of which may be requested from the Agency. When all the present "data gaps" have been filled and the submitted data have been reviewed, the Agency will revise the Registration Standard. Thereafter, when the Agency determines that the internally maintained addenda have significantly altered the conditions for registration under the Standard, the document will be updated and re-issued.

While the Registration Standard discusses only the uses and hazards of products containing the designated active ingredient(s), the Agency is also concerned with the potential hazards of some inert ingredients and impurities. Independent of the development of any one Standard, the Agency has initiated the evaluation of some inert pesticide ingredients. Where the Agency has identified inert ingredients of concern in a specific product to which the Standard applies, these ingredients will be pointed out in the Guidance Package.

Chapter II

Agency Position on Methoprene

Introduction

This chapter describes in detail the Agency's regulatory position on products which contain methoprene as the sole active ingredient, and also considers the use patterns of products that contain methoprene as well as other active ingredients. However, the regulatory position contained in this Standard reflects a review only of the active ingredient methoprene. The regulatory position adopted by the Agency incorporates a number of considerations. Among these condiderations are risk criteria found in Section 162.11 (a) of Title 40 of the U.S. Code of Federal Regulations and the need for additional data and/or label restrictions. The Agency's determination is presented below, and the rationale for this basic decision follows the position.

In addition to a decision on the registration of the active ingredient and rationale for this decision, this chapter includes the following: criteria for the registration of methoprene products under the Standard; acceptable ranges and limits for product composition, acute toxicity, and use pattern/application method; required labeling; tolerance reassessment.

The scientific basis for a decision presented in this chapter can be found by reading the various disciplinary chapters (Chapters IV-IX) which provide summaries of available scientific data on methoprene. The data requirements and data gaps are presented in Chapter III, Tables A-C. Also, the reason for establishing a data requirement can be found in the footnotes to the tables in Chapter III and also in the topical discussions portion of Chapters IV-IX. References to Agency guidelines for testing are provided when appropriate.

Description of Chemical

Methoprene is an insect growth regulator (larvicide) federally registered for use in feed/mineral supplements for cattle, on rice, pastures, stored tobacco, mushroom cultures, in and around homes, and on non-crop aquatic areas to control the horn fly, mosquito, cigarette beetle, tobacco moth, sciarid fly and flea larvae. A petition for use on peanuts has recently been reviewed and accepted. Several thousand pounds of the active ingredient methoprene are produced annually in the U.S. The percentages of methoprene sold domestically for specific uses are: 35% for mosquito control, 57% for horn fly control in combination with cattle mineral supplements, 2-3% for cigarette beetle control on tobacco, and 1-2% for control of mealy bugs and spider mites on houseplants. Exports of methoprene account for a few hundred pounds and are used primarily for research purposes.

Technical methoprene is a pale yellow liquid with a faint fruity odor. Technical methoprene is intended only for re-formulation into an end-use pesticide and, therefore, is considered a "manufacturing-use" product.

Methoprene is the common name for the chemical isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4,-dodecadienoate. Methoprene is also known by the trade name Altosid and other names, ZR-515 and ENT-70460. The Chemical Abstracts Registry (CAS) number for methoprene is 40596-69-8 and the EPA Shaughnessy number is 105401.

Regulatory Position for Products Containing Methoprene

Methoprene, isopropyl (2,4)-methoxy-3,7,11-trimethyl-2,4-dodecadienoate, as described in this Standard may be registered for sale, distribution, reformulation, and use in the United States. Considering all information available to the Agency from the open literature and provided to the Agency as of September 30, 1980, and all data submitted to the Agency by registrants, as of October 7, 1981, the Agency finds that none of the risk criteria found in Section 162.11(a) of Title 40 of the U.S. Code of Federal Regulations were met or exceeded for pesticide products containing methoprene.

The data base available for methoprene is sizeable and few data gaps exist. A review of the data base indicates that the use of methoprene will not result in unreasonalble adverse effects to man or the environment. Currently, registered methoprene products may be reregistered subject to the conditions imposed. New products and new uses may be registered under this Standard and are subject to the same requirements.

Regulatory Rationale

Methoprene is an insect growth regulator (larvicide) registered to control the horn fly, mosquito, cigarette beetle, tobacco moth, sciarid fly and flea larvae. Methoprene products are registered for both domestic and nondomestic, terrestrial/aquatic (food and non-food), indoor/outdoor use.

There are several methoprene products that are mixtures; that is, methoprene is not the sole active ingredient in the product. These products are registered for use on houseplants as insect and mite mists. The toxicology data base on methoprene supports the use of methoprene products in and around the home. Since products with methoprene as the sole active ingredient are registered for use in and around the home, the use pattern for the mixtures is not unique.

Few data gaps exist in the product chemistry data base. Data on the manufacturing processes of methoprene have been submitted by the registrants as well as documentation of the analytical methods for the identification and quantification of methoprene in the technical material and formulated end-use products. The physical and chemical properties for the technical material have been reported and color, odor, physical state and storage stability has been reported for most of the formulated products.

The toxicology data base for methoprene is almost complete. Acute toxicity data indicate low toxicity potential to humans. Methoprene is not a primary eye or dermal irritant and it is not anticipated to cause dermal sensitization. There are no chronic, oncogenic, mutagenic, teratogenic, or reproductive hazards associated with the chemical as indicated by experimental data. The metabolism of methoprene in several animal species has been documented.

The files of the Pesticide Incident Monitoring System (PIMS) contained a record of one incident involving accidental human exposure to methoprene. In this case, a 2 1/2 year old boy remained asymptomatic after methoprene, in a pressurized container, got into his mouth. Also, Zoecon Corporation reported 40 incidents involving accidental methoprene exposure to male chemical workers.

The incidents involved 26 exposures to technical methoporene and 14 exposures to a formulated methoprene product. Exposure to the technical product was through dermal contact in 12 out of 14 cases and via vapors in the other two cases. In all cases, no overt signs of toxicity were observed. Treatment consisted of washing the chemical off with soap and water. No medical treatment was indicated. In addition, the Zoecon report cited two other incidents involving exposure to methoprene. In one incident, a male plant operator was coated witth methoprene on the forehead, neck, forearms, and hands following an explosion. The victim did not experience any dermal or clinical signs of toxicity after washing with soap. The other incident involved a male worker who experienced no ill effects after extended dermal and inhalation exposure to technical methoprene.

Methoprene is not highly toxic to birds, mammals, or fish. Methoprene is somewhat more toxic to amphibians than to fish, but the low application rates and rapid dissipation characteristics of methoprene should prevent aquatic concentrations from reaching levels lethal to most amphibians. The use of methoprene as a mosquito larvicide poses some hazards to freshwater invertebrates, but major effects are unlikely. Laboratory studies found that methoprene is highly toxic to Daphnia magna, but only moderately toxic to a wide variety of other invertebrate species.

Marine organisms are not likely to be exposed to methoprene. Estuarine organisms, however, are likely to be exposed. The hazards to estuarine vertebrates as a result of the use of methoprene as a mosquito larvicide is expected to be slight because methoprene dissipates and degrades rapidly. Although the hazards from methoprene are greater for estuarine invertebrates than to vertebrates, the effects on estuarine invertebrates are not expected to be severe.

The use of methoprene as a mosquito larvicide should have no adverse effects on populations of endangered birds, mammals, or fish. If methoprene were used in a critical habitat area of one of these types of organisms, its low toxicity should prevent the loss of any endangered organisms.

Methoprene might pose hazards to populations of endangered amphibians and aquatic invertebrates. At present only one aquatic invertebrate, the Socorro isopod (Exosphaeroma thermophilus), is listed as endangered. The Socorro isopod is confined to a single stream in Socorro County, New Mexico. Its critical habitat is, therefore, very well defined, and applications of any pesticide in the area are unlikely.

The habitats of two endangered amphibians, the Houston toad (Bufo houstonensis) and the pine barrens tree frog (Hyla andersonii) are not defined. These two species of amphibians lay their eggs in habitats that are likely to be treated with mosquito larvicides. Therefore, the larvae of these amphibians could be exposed to methoprene applications. Methoprene is highly toxic (96-hr. LC_{50}=0.17ppm) to the larvae of the Fowler's toad (Bufo woodhousei), a species of the same genus as the Houston toad. Expected residues of methoprene would exceed the LC_{10} of methoprene to the larvae of the Fowler's toad. Therefore, methoprene could pose a risk to the Houston toad, if the pesticide were used within the critical habitat of this organism. No toxicity data were available for members of the genus, Hyla, but the high toxicity of methoprene to Bufo woodhousei raises concerns about the possible effects on the pine barrens tree frog. At the present time, a complete assessment of the hazards of methoprene to these endangered species cannot be made due to insufficient information. A complete hazard assessment for endangered species will be done pending receipt of further information and consultation with the Office of Endangered Species. At this time, the labels of all registered methoprene products intended for aquatic use should be modified to contain precautionary statements to prevent inadvertent introduction of methoprene into waters containing populations of aquatic invertebrates. The specific statements are listed in the Required Labeling section of this chapter.

No data gaps have been identified in the residue chemistry data base, considering currently registered use patterns. The metabolism of methoprene on plants and animals is adequately documented. The residue of concern is methoprene *per se*. Adequate methods of determining residues of methoprene in various commodities exist. Residue studies reflecting the registered uses of methoprene on raw agricultural crops, by-products and feed items are acceptable. The established tolerances for residues of methoprene are found to be adequate based on the evaluation of the residue data.

Criteria for Registration Under this Standard

To be subject to this Standard, methoprene products must meet the following conditions:

1. contain methoprene as an active ingredient;
2. be within acceptable standards of product composition;
3. be within acceptable acute toxicity limits;
4. be labeled for acceptable end-uses; and
5. bear required labeling.

Manufacturing-use methoprene products must bear label directions for formulation into acceptable end-uses.

The applicant for registration or reregistration of methoprene products subject to the Standard must comply with all terms and conditions described in this Standard, including commitment of fill data gaps on a time schedule specified by the Agency and, when applicable, offer to pay compensation to the extent required by 3(c)(1)(D) and 3(c)(2)(D) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended, 7 U.S.C. 136(c)(1)(D) and 136(c)(2)(D). As discussed in Chapter 1, applicants for the registration of methoprene products under this Standard must contact the Agency for specific instructions, including updated information on data requirements and companies whose data must be cited and to whom compensation must be offered.

A. Manufacturing-use Methoprene

1. Acceptable Ranges and Limits

a. Product Composition Standards

There are four currently registered manufacturing-use methoprene products: a technical methoprene product (90%), a manufacturing-use product (80%), and two formulation intermediate products (15% and 10%). To be covered under this Standard, manufacturing-use methoprene products must contain methoprene as the sole active ingredient or in combination with other active ingredients. Manufacturing-use methoprene products with any percentage of active ingredient with appropriate certification of limits are acceptable under this Standard.

b. Acute Toxicity Limits

The Agency will consider registration of manufacturing-use methoprene products which have established acute toxicity category I-IV ratings for each of the following effects:

Acute Oral Toxicity
Acute Dermal Toxicity
Acute Inhalation Toxicity
Primary Eye Irritation
Primary Dermal Irritation

c. Use Patterns

To be covered under this Standard, manufacturing-use methoprene products must be labeled for formulation into end-use pesticides which are intended for indoor/outdoor, domestic/nondomestic, terrestrial/aquatic (food or non-food uses), or other uses accepted by the Agency.

2. Required Labeling

All manufacturing-use methoprene products must bear appropriate labling as specified in 40 CFR 162.10.

3. Tolerance Reassessment

Methoprene, in plants, degrades to six principal non-polar metabolities: ZR-725, ZR-1945, ZR-669, ZR-1602, ZR-1564 and ZR-724. In animals, no additional non-polar metabolites were found. Methoprene is catabolized to 2-^{14}C-acetate which is a precursor of cholesterol in the animal.

C

An adequate, validated analytical method (gas-liquid chromatography) is available for determining residues of methoprene in commodities.

Tolerances for residues of methoprene have been established and published for the following food crops (40 CFR 180.339 as amended in FR Vol. 46 No. 233, Friday, December 4, 1981): milk (0.05 ppm); mushrooms (1.0 ppm); fat of cattle, goats, hogs, horses, sheep (0.3 ppm); meat and meat by-products of cattle, goats, hogs, horses, sheep (0.1 ppm); poultry fat and poultry meat (0.5 ppm); poultry meat by-products (0.05 pp,).

The toxicology data base used to support the tolerances on food crops is presented and discussed in the Toxicology Chapter of this Standard (Chapter VI). An 18-month feeding study in Charles River CD-1 mice was used to establish a NOEL of 250 ppm. This finding was also observed in some pigmentation of the liver at 2500 ppm. This finding was also observed in some mice at 1000 ppm, but was absent at the 250 ppm level and in the control group. In addition, a higher incidence of liver necrosis was observed in the females of the 5000 ppm group when compared to the control group.

Assuming residues in crops are at tolerance level, the theoretical maximum residue contribution (TMRC) is calculated to be 0.0853 mg/day (assuming a 1.5 kg diet). From these calculations, a diet of 1.5 kg contains 3.8% of the acceptable daily intake (ADI) of methoprene.

A request for an experimental use permit (EUP) to ship 200 pounds of the active ingredient methoprene as PZ-515-2, for control of stored product pests was recently received by the Agency. Also, a petition to establish a temporary tolerance of 10 ppm methoprene in or on the following stored commodities was recently received: dried beans, nuts (excluding peanuts), apples, raisins, peaches, pears, dried peas, cocoa/coffee beans. The petition requested the establishment of a temporary food additive tolerance of 10 ppm methoprene in or on the stored commodities: wheat flour, macaroni products from wheat, rice cereal, rye cereal, dog food (dry), wheat cereal, corn meal/grits and hominy, cereal from oats, and spices.

This petition is currently being reviewed by the Toxicology Branch.

B. End-use Methoprene: Emulsifiable Concentrate, Granular and Pelleted/Tableted (including mineral blocks), Pressurized Liquid

1. Acceptable Ranges and Limits

a. Product Composition Standards

End-use methoprene products with any percentage of active ingredient are acceptable under this Standard with appropriate certification of limits.

Inert ingredients in formulations used on food and food crops must be cleared for such use under 40 CFR 180.1001. All of the inert ingredients used in methoprene end-use products are cleared.

b. Acute Toxicity Limits

The Agency will consider registration of any end-use methoprene products for use with acute toxicity category ratings as listed in the table below:

	I	II	III	IV
Acute Oral Toxicity	amendment required	yes	yes	yes
Acute Dermal Toxicity	amendment required	yes	yes	yes
Acute Inhalation Toxicity	amendment required	yes	yes	yes
Primary Eye Irritation	amendment required	yes	yes	yes
Primary Dermal Irritation	amendment required	yes	yes	yes

c. Use Patterns

To be registered under this Standard, end-use products of methoprene must be labeled as an insect growth regulator (larvicide) to be used on one or more of the following: goats, hogs, sheep, poultry, horses, cattle, rice, pastures, peanuts, stored tobacco, mushroom cultures, in and around homes, and noncrop aquatic areas.

2. Required Labeling

All end-use methoprene formulations must bear appropriate labeling as specified in 40 CFR 162.10.

Additional Restrictions and Precautionary Statements

Although methoprene is highly toxic to some freshwater, estuarine, and marine invertebrates, none of the registered labels make any statement about the toxicity of the product. To prevent inadvertent introduction into waters containing populations of aquatic invertebrates, the labels of all product not intended for aquatic use should contain this statement.

> This product is toxic to aquatic invertebrates. Do not contaminate aquatic systems by cleaning of equipment or disposal of wastes.

The labels of products intended for aquatic use should contain this statement:

> This product is toxic to aquatic invertebrates. Using it in a manner other than those described by the label could result in harm to aquatic invertebrates.

Chapter III

Data Requirements and Data Gaps

Manufacturing-Use Methoprene

Table A entitled, Generic Data Requirements includes those data that pertain to the properties or effects of methoprene as an active ingredient. Thus, these data are relevant to an evaluation of the risks and benefits of all prodcuts containing methoprene. Providing data to fill indicated gaps is the primary responsibility of the manufacturing-use registrant. Registrants of end-use products which are not exempted by FIFRA Section 3(c)(2)(D) are also responsible for the submission of these data. Applicants for the registration or reregistration of manufacturing-use methoprene products must acknowledge reliance on existing data which fill indicated data requirements under FIFRA 3(c)(1)(D). These data are listed under the column entitled Bibliographic Citation in this table. All data requirements are required for all currently registered use patterns unless a footnote to the table specifies otherwise.

Table B, entitled Product-Specific Data Requirements for Methoprene Manufacturing-Use Products, includes those data that relate only to the properties or effects of a product with a specific composition. Thus, these data are required of each product to characterize the product's particular composition and physical/chemical properties, and acute toxicity. Providing data to fulfill these data requirements for a particular product is the responsibility of each applicant for the registration or reregistration of a manufacturing-use methoprene product. If the Agency has in its possession product-specific data which fulfill a data requirement for a particular product, this is indicated in the guidance package accompanying this Standard.

Applicants for the registration of new manufacturing-use methoprene products must submit all required product-specific data or establish that the proposed product is substantially similar to another product for which the Agency has received acceptable product-specific data.

If the Agency has determined that one or more existing manufacturing-use methoprene products are substantially similar, then this, too, is indicated. Product-specific data need not be acknowledged under FIFRA 3(c)(1)(D) unless the Agency or a registrant has established that a product is substantially similar to another product for which the Agency has received acceptable product-specific data. If this should occur, the registrant(s) of the former product(s) is required to acknowledge reliance on these data.

End-Use Products of Methoprene

Registrants of end-use methoprene products not exempt by FIFRA Section 3(c)(2)(D) are responsible for the submission of "generic" data described in Tables A and B of this Chapter, in addition to the product-specific data listed in Table C.

Registrants of all end-use methoprene products are advised that if data are not generated to fill generic data requirements for the manufacturing-use product(s), these registrations will be suspended. If continued availablility of the manufacturing-use product is desired, these data must be supplied.

Table C, entitled Product-Specific Data Requirements for Methoprene End-Use Products, includes those data that relate only to the properties or effects of an end-use product with specific composition.

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Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Does EPA Have Data to Partially or Totally Satisfy Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, deadline for submission
Product Chemistry					
163.61-3(b)	Identification	Yes	Yes	1/	No
163.61-8(1)	Color	Yes	Yes	Zoecon, 1972, 00010927	No
163.61-8(2)	Odor	Yes	Yes	Zoecon, 1972, 00010927	No
163.61-8(3)	Melting Point	No			
163.61-8(4)	Solubility	Yes	Yes	Thalheimer, 1979, 00010925	No
163.61-8(5)	Stability	Yes	Yes	Zoecon, 1972, 00010927	No
163.61-8(6)	Octanol/Water Partition coefficient	Yes	No	-	Yes: 6-8 months
163.61-8(7)	Physical State	Yes	Yes	Zoecon, 1972, 00010927	No

1/ Data were obtained from the most recent confidential statement of formula submitted.

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Table A

Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Does EPA Have Data to Partially or Totally Satisfy Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, deadline for submission
Product Chemistry					
163.61-8(8)	Density or Specific Gravity	Yes	Yes	Zoecon, 1972, 00010927	No
163.61-8(9)	Boiling Point	Yes	Yes	Zoecon, 1972, 00010927	No
163.61-8(10)	Vapor Pressure	Yes	Yes	Zoecon, 1972, 00010927	No
163.61-8(11)	pH	No2/	Yes	Zoecon, 1972, 00010927	No

2/ Although data on the pH of methoprene are available, these data are not required since methoprene is practically insoluble in water.

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Table A

Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Composition	Data EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Environmental Fate:						
163.161-1	Hydrolysis	Yes	Radiolabeled analytical grade or nonlabeled technical grade	Yes	Schooley and Creswell, 1972, 00010439	No
163.161-2	Photodegradation in water	Yes	Radiolabeled analytical grade or nonlabeled technical grade	No	-	Yes: 8 months
163.161-3	Photodegradation in soil	No				
163.161-4	Photodegradation in air	Yes1/	Radiolabeled analytical grade or nonlabeled technical grade	No	-	Yes: 8 months1/

1/ These data will be required by the Agency depending upon the results and evaluation of data from laboratory volatility tests (Section 163.163-2(b)(2)).

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Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to partially or Totally Satisfy this Requirement?	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Environmental Fate:					
163.162-1	Aerobic Soil	Yes	Radiolabeled analytical grade or nonlabeled technical grade	No	Yes: 18 months
163.162-2	Anaerobic Soil	No			
163.162-3	Anaerobic Aquatic	Yes	Radiolabeled analytical grade or nonlabeled technical grade	No	Yes: 18 months
163.162-4	Aerobic Aquatic	Yes	Radiolabeled analytical grade or nonlabeled technical grade	No	Yes: 18 months

Table A

Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Environmental Fate:						
163.62-8(f)	Effects of microbes on pesticides	Reserved2/				
163.62-8(f)	Effects of pesticides on microbes	Reserved2/				
163.62-8(g)	Activated sludge metabolism	Reserved2/				
163.163-1	Leaching	Yes	Radiolabeled analytical grade or nonlabeled technical grade	No		Yes: 8 months
163.163-2	Laboratory volatility	Yes	Typical end use product of a major formulation category	No		Yes: 8 months
163.163-3	Field volatility	Yes1/	Typical end-use product of a major formulation category	No		Yes: 8 months

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1/ These data will be required by the Agency depending upon the results and evaluation of data from laboratory volatility tests.

2/ The requirement for these data is reserved pending the development and modification of test protocols.

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Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Environmental Fate:						
163.164-1	Field Dissipation Terrestrial	Yes	Representative formulation from major formulation categories	No		Yes: 12 months
163.164-2	Field Dissipation Aquatic and Aquatic Impact	Yes	Representative formulation from major formulation categories	Yes	3/	No
163.164-3	Dissipation-Forestry	No				
163.164-4	Dissipation-combination and Tank Mixes	No				
163.164-5	Dissipation-Long Term Studies	Yes4/	Representative formulation from major formulation categories	No		Yes: 24 months4/

3/ Schaefer and Dupras, 1973, 05008625, Steelman, 1972, 00011485, Hazeltine, 1972, 00012729; Schaefer, 1972, 00010434; 00010435, 00010436, Schaefer, 1972, 00010437, Kauffman, 1972, 00010438; Zoecon, 197?, 00011484; Steelman, 1972, 00010433; Zoecon, 1972, 00010417; Biedler, 1977, 00011091, 00011092;

4/ Required only if dissipation rate shown by field dissipation (terrestrial) tests is less than 50%.

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Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Environmental Fate:						
163.165-1	Confined Accumulation-Rotational Crops	Yes	Radiolabeled analytical grade of active ingredient	No	-	Yes: 24 months
163.165-2	Field Accumulation-Rotational Crops	Yes	Representative end-use product of major formulation category	No	-	Yes: 36 months5/
163.165-3	Accumulation-Irrigated Crops	No	-	-	-	-
163.165-4	Laboratory Studies - Fish	Yes	Radiolabeled analytical grade or technical grade active ingredient	No	-	Yes: 8 months 6/
163.165-5	Field Accumulation Aquatic Non-target	Yes	Representative end use product of major formulation category	No	-	Yes: 8 months7/

5/ Required only when significant C^{14} residues are found, Section 163.165-1, or when subsequent crop is treated with the same active ingredient as initial crop.

6/ Fish accumulation data will not normally be required in situations where the registrant can offer acceptable evidence showing that the active ingredient and/or its principal degradation products(s):
- o Will not reach water, or
- o Will not persist in water (i.e, a half-life of approximately 4 days or less) and has properties suggesting:
  - A relatively low potential for accumulation in fish (i.e., and octanol/water partition coefficient less than 1,000) or
  - A lack of accumulation in the organs and tissues of mammals or birds.

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Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Does EPA Have Data to Partially Composition	or Totally Satisfy this Requirement?	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission Bibliographic Citation	from published date of standard
Environmental Fate:						

7/ Field accumulation studies in aquatic non-target organisms are required to support the registration of each end-use product:
  - o Which is intended for forestry use, aquatic non-crop use, or aquatic impact use that results in direct discharges;
  - o For which data from a laboratory fish accumulation study are required by Section 163.165-4; and
  - o For which no tolerance or action level for fish has been granted.

Table A

Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Toxicology:						
163.81-1	Acute Oral Toxicity	Yes	Technical Grade	Yes	Hallesy *et al.*, 0024607	No
163.81-2	Acute Dermal Toxicity	Yes	Technical Grade	Yes	Hepler *et al.*, 00010914	No
163.81-7	Acute Neurotoxicity	No				
163.82-1	Subchronic Oral Toxicity	Yes	Technical Grade	Yes	Jorgenson, 1972, 00024612	No
163.82-2	Subchronic 21-day Dermal Toxicity	Yes	Technical Grade	Yes	Zoecon, 1977, GS0030-0011	No
163.82-3	Subchronic 90-day Dermal Toxicity	No				
163.83-4	Subchronic Inhalation Toxicity	No				

Table A

Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Toxicology:						
163.83-1	Chronic Feeding	Yes	Technical Grade	Yes	Wazeter, 1974, 00010739	No
163.83-2	Oncogenicity	Yes	Technical Grade	Yes	Wazeter, 1974, 00010739 Wazeter, 1975, 00010600	No
163.83-3	Teratogenicity	Yes	Technical Grade	Yes	Nomura, 1975, GS0030-0006	No
163.84-2 through 4	Mutagenicity	Yes	Technical Grade	Yes	Hsia et al., 1979, 05018270 Johnston, 1973, 00010545	No

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Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Toxicology:						
163.85-1	Metabolism	Yes	Technical Grade	Yes	Chausseaud, 1974, 0010866 Hawkins, 1977, 05007755 Zoecon, 1972, 0010425 Cline, 1972, 00010424 Chamberlain, 1973, 0010379, 00010380 Zoecon, 1973, 00011491 Staiger, 1973, 00010879 Quistad, 1975, 05008609 Zoecon, 1974, 00010681 Quistad 1974, 000010626 Chamberlain, 1974, 00010683	No

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Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Residue Chemistry:						
	Metabolism in Plants	Yes		Yes	1/	No
	Metabolism in Animals	Yes		Yes	2/	No
	Analytical Methods	Yes		Yes	3/	No
	Residue Data: Crops	Yes		Yes	4/	No
	Residue Data: Meat and Milk	Yes		Yes	5/	No
	Storage Stability	Yes		Yes	6/	No

1/ Quistad, 1974, 05007756, Zoecon, 1978?, 00011031, Zoecon, 1973, 00010875, Zoecon, 197?, 00010422, Staiger, 197? 00010369, Staiger, 1972, 00010368; Zoecon, 1973, 00010366; Schooley, 1975, 05008622;

2/ Zoecon, 1974, 00012761; Chamberlain, 1974, 00010682; Quistad, 1974, 00010684; Quistad, 1974, 00010867; Zoecon, 1974, 00011098; Zoecon, 197?, 00010692; Quistad, 1974, 00011099;

3/ Hunt, 1975? 00011105; Miller, 1974, 00010687; Miller, 1973, 00010938; Zoecon, 197?, 00011490; Miller, 1975, 00026940;

4/ Washino, 1973, 00010827; Meisch, 1973, 00010374; Meisch, 1973, 00012782; Zoecon, 1973, 00011493; Meisch, 1973, 00010374; Meisch, 1973, 00012782, Zoecon, 1973, 00012787; Zoecon, 1979, 00012796; Markowitz, 197?, 00001240; Hunt, 1976, 05007751

5/ Chamberlain, 1974, 00010682; Zoecon, 197?, 00010894; Zoecon, 197?, 00010898; Miller, 1973, 00010832; Zoecon, 197?, 00010897; Moorman, 197?, 00010619; Kearley, 1975, 00010896; Zoecon, 1976, 00010889; Zoecon, 197? 00010895; Zoecon, 1974, 00010680; Zoecon, 1973?, 00012783, Wright, 1973, 00010833; Zoecon, 1974?, 00010893;

6/ Since there is no significant time interval between samplingand analysis, no storage stability data are required.

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Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Ecological Effects						
163.71-1	Avian Single-Dose Oral LD_{50}	Yes	Technical Grade	Yes	Hudson, 1975, 00010633	No
163.71-2	Avian Dietary LC_{50}	Yes	Technical Grade	Yes	Fink, 1972, 00012754 Fink, 1972, 00012755	No
163.71-3	Wild Mammal Toxicity	No				
163.71-4	Avian Reproduction	Yes	Technical Grade	Yes	Fink & Reno, 1973, 00010634 Fink & Reno, 1973, 00010635	No
163.71-5	Simulated and Actual Testing for Mammals and Birds	No				

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Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Ecological Effects						
163.72-1	Fish Acute LC_{50}	Yes	Technical Grade	Partial	Sleight, 1972, 0001038	Yes: 6 months[1/]
163.72-2	Aquatic Invertebrate Acute EC_{50}	Yes	Technical Grade	Yes	LeBlanc, 1975, 00010856	No
163.72-3	Acute Toxicity to Estaurine and Marine Organisms	Yes	Technical Grade	Yes	Sleight, 1972, 00010851 Sleight, 1973, 00010852 Costlow, 1975, 05010827 Costlow, 1977, 00010860 Petrocelli, 1975, 00010859	No
163.72-4	Fish Early Life-Aquatic Invertebrate life-Cycle	Reserved[2/]	Technical Grade	No		No

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[1/] Data on the static acute toxicity of methoprene to warm water fish have been received. Data on the acute toxicity of methoprene to cold water fish (preferably the rainbow trout) are needed.

[2/] These tests may be necessary in order to complete an accurate hazard assessment. The requirement for submission of these data is being reserved pending receipt of the other ecological effects data and environmental fate data.

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Table A

Generic Data Requirements for Methoprene

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Ecological Effects						
163.72-5	Fish Life-Cycle	Reserved[2/]	Technical Grade	No	-	
163.72-6	Aquatic Organism Accumulation	Yes	Technical Grade	Yes	Quistad et al., 1976, 05008611 Sleight, 1973, 00010903 Staiger et al., 1977, 00010390	No
163.72-7	Simulated or Actual Field Testing for Aquatic Organisms	Reserved[2/]	Technical Grade	No	-	
163.70-1(d)	Reptile and amphibian LC_{50}	Yes	Technical Grade	Yes	Cliburn, 1973,	No
163.122-1	Seed Germination	Yes	Technical Grade	No	-	No[3/]
163.122-1	Vegetative Vigor	Yes	Technical Grade	No	-	No[3/]
163.122-2	Algae	Yes	Technical Grade	No	-	No[3/]
162.122-2	Aquatic macrophytes	Yes	Technical Grade	No	-	No[3/]

[2/] These tests may be necessary in order to complete an accurate hazard assessment. The requirement for submission of these data is being reserved pending receipt of the other ecological effects data and environmental fate data.

[3/] These data requirements have been waived for methoprene.

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Tab

Product-Specific Data Requirements for Manufacturing-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, deadline for submission from published date of standard
Product Chemistry:					
163.61-3	Product Identity and Disclosure of Ingredients	Yes	Yes	Zoecon, 1973?, 00012800 Zoecon, 1972, 00011495 Zoecon, 1973, 00011482	No
163.61-4	Description of Manufacturing Process	Yes	Yes	Zoecon, 19??, 00010930 Zoecon, 1974, 00010656 Zoecon, 1974?, 00010482	No
163.61-5	Discussion of Formation of Unint. Ingredients	Yes	Yes	Zoecon, 19??, 00010973 Zoecon, 19?? 00010808	No
163.61-6	Declaration and Certification of Ingredient Limits	Yes	Yes	Zoecon, 1973, 00011482	No

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Ta B

Product-Specific Data Requirments for Manufacturing-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, deadline for submission from published date of standard
Product Chemistry:					
163.61-7	Product Analytical Methods and Data	Yes	Yes	Dunham, 1978, 05013295 Zoecon, 19??, 00010699 Moorman, 1975, 00010621 Rhoades, 1977, 00010907 McFall-Quistad, 1975, 00010883 Lowe, 19?? 00010665 Thalheimer, 1979, 00010925 Wenik, 19??, 00010933 Nicoara, 1976, 00011103	No
163.61-8(c)(7)	Physical State	Yes	Yes	Zoecon, 1972, 00010927	No
163.61-8(8)	Density or Specific Gravity	Yes	Yes	Zoecon, 1972, 00010927	No
163.61-8(9)	Boiling Point	Yes	Yes	Zoecon, 1972, 00010927	No

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Table B

Product-Specific Data Requirements for Manufacturing-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required	Does EPA Have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, deadline for submission from published date of standard
Product Chemistry:					
163.61-8(c)(11)	pH	Yes	Yes	Zoecon, 1972, 00010927	No
163.61-8(c)(12)	Storage Stability	Yes	Yes	Dunham, 1978, 05013295	No
163.61-8(c)(13)	Flammability	Yes	Yes	Zoecon, 1972, 00010803	No
163.61-8(c)(14)	Oxidizing or Reducing Action	Yes	Yes	Dunham, 1978, 05013295	No
163.61-8(c)(15)	Explosiveness	No			
163.61-8(c)(16)	Miscibility	Yes	No		Yes: 6-8 months
163.61-8(c)(17)	Viscosity	Yes	No		Yes: 6-8 months
163.61-8(c)(18)	Corrosion Characteristics	Yes	Yes	Zoecon, 19??, 00012732	No

Table B

Product-Specific Data Requirements for Manufacturing-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Toxicology						
163.81-1	Acute Oral Toxicity	Yes	MUP	Partial	Hallesy et al, 1972, 00024607	Yes: 6 months[1]
163.81-2	Acute Dermal Toxicity	Yes	MUP	Partial	Hepler et al., 1979, 00010914	Yes: 6 months[1]
163.81-3	Acute Inhalation Toxicity	Yes	MUP	Partial	Olson & Willigan, 1972, GS0030-0009	Yes: 6 months[1]
163.81-4	Primary Eye Irritation	Yes	MUP	Partial	Hallesy & Hill, 1972, 00024614	Yes: 6 months[1]
163.81-5	Primary Dermal Irritation	Yes	MUP	Partial	Hallesy & Hill, 1972, 00024615	Yes: 6 months[1]
163.81-6	Dermal Sensitization	Yes	MUP	Partial	Zoecon, 1977, GS0030-0012	Yes: 6 months[1]

1/ Based on an evaluation of the inert ingredients, testing is required on the manufacturing-use product containing 15% methoprene.

2/ Based on an evaluation of the inert ingredients, testing with each existing product is required.

3/ This requirement has been waived for all methoprene products except the MUP containing 15% methoprene. Testing on this product is needed based on an evaluation of the inert ingredients.

Table B

Product-Specific Data Requirements for Manufacturing-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Product Chemistry						
163.61-3	Product Identity and Disclosure of Ingredients	Yes	Each product	Yes	-	No[1/]
163.61-4	Description of Manufacturing Process	Yes	Each product	No	-	Yes: 6-8 months
163.61-5	Discussion of Formation of Unint. Ingredients	Yes	Each product	Yes	Zoecon, 19??, 00010973 Zoecon, 19??, 00010808	No[2/]
163.61-6	Declaration and Certification of Ingredient Limits	Yes	Each product	Yes	-	No[1/]

1/ Data were obtained from the latest registered label.

2/ Impurities in the end-use formulated product are the same as those found in the bulk manufacturing-use product; their proportion corresponds to the methoprene concentration in the end-use formulation.

Table C

Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Product Chemistry						
163.61-7	Product Analytical Method & Data	Yes	Each product	Yes	Dunham, 1978, 05013295 Zoecon, 19??, 00010699 Moorman, 1975, 00010621 Rhoades, 1977, 00010907 McFall-Quisted, 1975, 00010883 Lowe, 19??, 00010665 Thalheimer, 1979, 00010925 Wenik, 19??, 00010933 Nicoara, 1976, 00011103 Zoecon, 19??, 00010971 Wenik, 1973 00010806	No

Table C

Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Product Chemistry						
163.61-8(c)(1)	Color	Yes	Each product	Yes	1/	No
163.61-8(c)(2)	Odor	Yes	Each product	Yes	1/	No
163.61-8(c)(3)	Melting Point	No				
163.61-8(c)(4)	Solubility	No				
163.61-8(c)(5)	Stability	No				
163.61-8(c)(6)	Octanol/Water	No				
163.61-8(c)(7)	Physical State	Yes	Each product	Yes	1/	No

1/ Zoecon, 1972, 00010803; Zoecon, 19??, 00010908; Zoecon, 19??, 000010661, Cunningham, 19??, 00020074; Moorman, 1974?, 00010354; Zoecon, 1976, 00012738; Zoecon, 19??, 00010885; Zoecon, 1974, 00011492; Zoecon, 00010516; Moorman, 1974, 00010596; Zoecon, 1976, 00010488; Zoecon, 19??, 00010501; Zoecon, 1978, 00010905; Zoecon, 19??, 00010460; Zoecon, 19??, 00010459; Zoecon, 19??, 00010662; Zoecon, 1977?, 00010461; Zoecon, 1974, 00010931; Zoecon, 1974, 000010932; Moorman, 1978?, 00010415; Zoecon, 19??, 00012732; Zoecon, 1974, 000010934; Zoecon, 1970?, 00011085; Zoecon, 1977?, 00011486; Zoecon, 1976, 00010483, Moorman, 1976?, 00010413.

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Product Chemistry						
163.61-8(c)(8)	Specific Gravity	Yes	Each product	No	-	Yes: 6 months
162.61-8(c)(9)	Boiling Point	No				
163.61-8(c)(10)	Vapor Pressure	No				
163.61-8(c)(11)	pH	Yes	Each product	No	-	Yes: 6 months
163.61.8(c)(12)	Storage Stability	Yes	Each product	Yes	1/	No
163.61-8(c)(13)	Flammability	No	Each product			

1/ Zoecon, 1972, 00010803; Zoecon, 19??, 00010908; Zoecon, 19??, 000010661, Cunningham, 19??, 00020074; Moorman, 1974?, 00010354; Zoecon, 1976, 00012738; Zoecon, 19??, 00010885; Zoecon, 1974, 00011492; Zoecon, 1977, 00010516; Moorman, 1974, 00010596; Zoecon, 1976, 00010488; Zoecon, 19??, 00010501; Zoecon, 1978, 00010905; Zoecon, 19??, 00010460; Zoecon, 19??, 00010459; Zoecon, 19??, 00010662; Zoecon, 1977?, 00010461; Zoecon, 1974, 00010931; Zoecon, 1974, 000010932; Moorman, 1978?, 00010415; Zoecon, 19??, 00012732; Zoecon, 1974, 00010934; Zoecon, 1970?, 00011085; 00020625; Zoecon, 1977?, 00011486; Zoecon, 1976, 00010483, Moorman, 1976?, 00010413.

Table C

Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Product Chemistry						
163.61-8(c)(14)	Oxidizing or Reducing Action	Yes	Each product	No	-	Yes: 6 months
163.61-8(c)(15)	Explosiveness	No				
163.61-8(c)(16)	Miscibility	Yes	Each product	No	—	Yes: 6 months
163.61-8(c)(17)	Viscosity	Yes	Each product	No	-	Yes: 6 months
163.61.8(c)(18)	Corrosion	Yes	Each product	No	-	Yes: 6 months

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Product Chemistry						
163.81-1	Acute Oral Toxicity	Yes	Each product	Yes	Hepler et al., 1979, 00010913 Hallesy et al., 1972, 00024608	No
163.81-2	Acute Dermal Toxicity	Yes	Each Product	Yes	Hepler et al, 1979, 00010914 Olsen, 1972, 00024618	No
163.81-3	Acute Inhalation Toxicity	Yes	Each Product	Yes	Olson and Willigan, 1972, GS0030-0009	No
163.81-4	Primary Eye Irritation	Yes	Each Product	Yes	Hepler, 1979, 00010915	Yes: 6 months[1/]
163.81-5	Primary Eye Irritation	Yes	Each Product	Yes	Hepler, 1979, 00010915	No
163.81-6	Dermal Sensitization	Yes	Each Product	No[2/]	-	No

1/ Testing with the 5% emulsifiable product is required.

2/ Based on an evaluation of the inert ingredients and the available acute data, dermal sensitization is not anticipated with these products. This data requirement is waived.

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Toxicology: Granular and Pelleted Tablets						
163.81-1	Acute Oral Toxicity	Yes	Each Product	Yes	Hallesy et al., 00024609	No
163.81-2	Acute Dermal Toxicity	Yes	Each Product	Yes	Hepler et al., 1979, 00010914	No
163.81-3	Acute Inhalation Toxicity	Yes	Each Product	Yes	Olson and Willigan 1972, GS0030-0009	No
163.81-4	Primary Eye Irritation	Yes	Each Product	No	- 00010915	Yes: 6 months[3/]
163.81-5	Primary Dermal Irritation	Yes	Each Product	Yes	Hallesy & Hill, 1972, 00024615	No
163.81-6	Dermal Sensitization	Yes	Each Product	No[4/]	-	No

3/ Testing with one granular formulated product is needed. The results of this test will fulfill testing requirements for the pelleted/tableted formulations also.

4/ Based on the available data for technical methoprene, dermal sensitization is not anticipated with these products. This data requirements is waived.

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Toxicology: Pressurized Liquid						
163.81-1	Acute Oral Toxicity	Yes	Each Product	No	-	No[5/]
163.81-2	Acute Dermal Toxicity	Yes	Each Product		-	No[5/]
163.81-3	Acute Inhalation Toxicity	Yes	Each Product	No	-	No[5]
163.81-4	Primary Eye Irritation	Yes	Each Product	No	-	Yes: 6 months[6/]
163.81-5	Primary Dermal Irritation	Yes	Each Product	No	-	No[5/]
163.81-6	Dermal Sensitization	Yes	Each Product	No	-	No[5/]

5/ Testing with the 15% methoprene manufacturing-use product will be sufficient to fulfill this data requirement for the pressurized liquid product.

6/ Testing is required on the 0.15% liquid product.

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Ecological Effects:						
163.72-1	Fish Acute LC_{50}	Yes	10%	Yes	Fink, 1972, - 00010640	No
163.72-4	Fish Early Life-Stage; Aquatic Invertebrate Life Cycle	Reserved[2/]	10%	No		
163.72-5	Fish Life-Cycle	Reserved[2/]	10%	No		
163.72-7	Simulated or Actual Field Testing for Aquatic Organisms	Reserved[2/]	10%	Yes	Takahashi & Miura, 1975 00010645	

2/ These tests may be necessary in order to complete an accurate hazard assessment. The requirement for submission of these data is being reserved pending receipt of the other ecological effects data and environmental fate data.

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Efficacy:						
Pastures	Aedes mosquitoes (larvae)	Yes	EC (0.86 lb/gal or unspecified)	Yes	Lawrence & Ramke, 1974, 05010838 Schaefer et al, 1978, 05010836 Wagstaf & Hinsow, 1974, 05015768 Schaefer; 1972 00011012; 1972, 00011011; 1972 00011010; 1973, 00010547; 1973, 00010524, 1973, 00010557 White, 1973, 00010532 Murray, 1973 00010525	No
	Culex Mosquitoes				Schaefer, 1972, 00010998; 1972, 00011002; 1972, 00010999	

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Efficacy: (Cont'd)						
Rice	Psorophora Mosquitoes (larvae)	Yes	EC (0.86 lb/gal or unspecified)	Yes	Steelman et al., 05009393 Steelman, 1973, 00010948; 1973, 00010949; 1973, 00010950 Meisch, 1973 00012782	No
	Aedes Mosquitoes (larvae)	Yes	4EC EC (0.86 lb/gal)	Yes	Steelman et al., 00010956; 1973, 00010955; Meisch, 1973 00012782	No
Catch Basins	Culex Mosquitoes (larvae)	Yes	Briquette	Yes	Cape May Mosquito Control Commission, 1977, 00011079	No
	(larvae)				Schoeppner, 1977, 000010514	
	Culiseta Mosquitoes (larvae)	Yes	4% Briquette		Stewart, 1977, 05009592 Schoeppner, 1977, 00010514	

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Efficacy: (Cont'd)						
Cesspools	Anopheles Mosquitoes (larvae) Culex Mosquitoes (larvae) floodwater complex of mosquito larvae	Yes	-	No	-	Yes: 12 months
Ditches	Aedes Mosquitoes (larvae)	Yes	4% Briquette Briquette - unspecified BC (0.86 lb/gal)	Yes	Stewart, 1977, 05009592 Forester, 197?, 00011049 Zoecon, 1975?, 00010960; West Florida Anthropod Research Laboratory, 1975, 00010966	
	Culex Mosquitoes (larvae)		BC (10F) 4% Briquette Briquette-unspecified		Self et al., 1978, 05008751 Stewart; 1977, 05009592 Forester, 197?, 00011049 Schoeppner, 1977, 00010514	

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Efficacy: (Cont'd)						
Ditches	Culiseta Mosquitoes (larvae)	Yes	Briquette - Unspecified	Yes	Schoeppner, 1977, 00010514 Forester, 19??, 00011049	No
Drainage	Floodwater complex of mosquito larvae	Yes	(See Catch Basins, Ditches, Pastures, Rice, Salt, and Tidal Marshes)			No
Dredging Spoil Sites	Floodwater complex of mosquito larvae	Yes	(See Catch Basins, Ditches, Pastures, Rice, Salt, and Tidal Marshes)			No
Estuarine Areas	Floodwater complex of mosquito larvae	Yes	(See Salt and tidal Marshes)			No

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Efficacy: (Cont'd)						
Intermittently Flooded Areas (including abandoned swimming pools, flooded crypts, natural and man made depressions and transformer vaults)		Yes				No
	Aedes Mosquitoes (larvae)		EC (0.86 lb/gal)		Buzicky, 1973, 00010528	
	Culex Mosquitoes (larvae)		0.028 g briquette		Dame, 1975, 00011057; 1975, 00012797	
			4% briquette		Mulla et al., 1977, 05000767	
			EC (0.86 lb/gal		Buzicky, 1973.	
	Culiseta Mosquitoes (larvae)		EC (0.86 lb/ gal) 4% briquette		Buzicky, 1973, 00010528 Mulla et al. 1977 05000767	

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Efficacy: (Cont'd)						
Marshes and Swamps	Aedes Mosquitoes (larvae)	Yes	EC (0.86% lb/ gal) Briquette unspecified Briquette (10F) Briquette unspecified EC (0.86 lb/ gal) EC (0.86 lb/ gal on sand)		Shaefer, 1972, 00011005 Forester, 19??, 00011049 Palmer, 1975, 00010453 Schoeppner, 1977, 00011050 Sjogren, 1976, 00011066 1976, 00011065 West Florida Anthropod Research Laboratory, 1975, 00010962 Shendahl, 1973, 00012740	No
	Culex Mosquitoes (larvae)		EC (0.86 lb/ gal) Briquette unspecified		Schaefer, 1972 00011005 Forester, 19??, 00011049 Sjogren, 1976, 00011065	

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Efficacy: (Cont'd)						
	Culiseta Mosquitoes (larvae)		Briquette (10F) Briquette unspecified		Palmer, 1975, 00010453 Forester, 1977, 00011049 Anon, 1977, 00011050	
Ornamental Ponds and fountains	Culex Mosquitoes (larvae)	Yes	4% Briquette (See Intermittent Flooded areas)	Yes	Stewart, 1977, 05009592	No
Roadside Ditches	Anopheles Mosquitoes Culex Mosquitoes (larvae) Culiseta Mosquitoes (larvae) Floodwater mosquito complex (larvae)		(See Ditches and Catch Basins)			

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Efficacy: (Cont'd)						
Salt and Tidal Marshes	Aedes Mosquitoes	Yes	EC unspecified	Yes	Rathburn and Boike, 1975, 05009634; 1977, 05009635 Rogers et al., 1976, 05008608 Sheldahl, 1974, 00010451	
			EC (SR-10 or SR-10F)		Spencer et al, 1979, 05020219 Vorgetts, 1976, 05010646	
			Briquette unspecified		Rathburn and Boike, 1977, 05009635 Sheldahl, 1974, 00010451; 00010452	
			EC (0.86 lb/gal)		Scheldahl, 1973, 00012779; 00010554 Sutherland, 1973, 00012778; 00012741; Uhrich, 1973?, 00010534 Anon, 1975, 00010962	

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Efficacy: (Cont'd)						
	Psorophora Mosquitoes (larvae)		EC (SR-10) and (SR-10F)		Spencer et al., 1979, 05020219	
	Floodwater Mosquitoes (larvae)		EC (SR-10) and (SR-10F)		Spencer et al., 1979, 05020219	
Septic Tanks	Anopheles Culex Culiseta Floodwater Complex of Mosquito (larvae)	Yes	4% Briquette	Yes	Stewart, 1977, 05009592	Yes: 12 months
Storm Drains	Culex & Culiseta Mosquito (larvae)	Yes	Briquette material formed into discs	Yes	Hazelrigg & Pelsue, 1979, 05020795	No

(See also Catch Basins & Roadside Ditches)

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Product-Specific Data Requirements for End-Use Methoprene Products

Guidelines Citation	Name of Test	Are Data Required?	Composition	Does EPA have Data to Partially or Totally Satisfy this Requirement?	Bibliographic Citation	Must Additional Data be Submitted under FIFRA 3(c)(2)(B)? If so, months allowed for submission from published date of standard
Efficacy: (Cont'd)						
Waste Treatment settling ponds	Anaopheles Culex Culiseta Floodwater Complex of Mosquito (larvae)	Yes	Briquette and (SR-10F)	Yes	McQueen, 1976, 00011067	Yes
Woodland Meadows	Floodwater complex of mosquito complex	Yes		No	(see Pastures and Woodland pools)	No
Woodland Pools	Aedes & Psorophora	Yes	EC (0.86 lb/gal	Yes	Rodrigues & Wright, 1978, 05009639 Baldwin & Chant, 1976, 05009909 Meisch, 1972, 00010991	No

Chapter IV

Product Chemistry

Introduction

FIFRA 3(c)(2)(A) requires the Agency to establish guidelines for registering pesticides in the United States. The EPA requires registrants to provide quantitative data on all active and added inert ingredients, and on those impurities which are equal to or greater than 0.1% of the product by weight.

To establish the composition of products proposed for registration, the Agency requires data and information not only on the manufacturing and formulation processes but also a discussion on the formation of manufacturing impurities and other product ingredients, intentional and unintentional. Further, to assure that the composition of the product as marketed will not vary from the composition evaluated at the time of registration, applicants are required to submit a statement certifying upper and lower composition limits for the added ingredients, and upper limits for some unintentional ingredients. Subpart D of the Proposed Guidelines (43 FR 29696, July 10, 1978) suggests specific precision limits for ingredients based on the percentage of ingredient and the standard deviation of the analytical method.

In addition to the data on product composition, the Agency guidelines also require data on the physical and chemical properties of both the pesticide active ingredient and formulations. For example, data are needed concerning the identity and physical state of the active ingredient such as melting and boiling point data, ambient vapor pressure and solubility. Data are also required on the properties of the formulated product to establish labeling precautions (e.g., flammability, corrosiveness, or storage stability). The Agency uses these data to characterize each pesticide and to determine its environmental and health hazards.

Product Chemistry: Manufacturing-use Methoprene

Product Chemistry Profile

Methoprene is the common name determined by the American National Standards Institute in 1973 for the chemical isopropyl (2E, 4E)-11-methoxy-3,7,11-trimethyl-2,4,-dodecadienoate. Methoprene is also known by the trade name Altosid and other names, ZR-515 and ENT-70460.

Technical methoprene is a pale yellow liquid with a faint fruity odor and has a boiling point of 100°C at 0.05 mm of Hg. Samples of methoprene stored in glass for 4 years at 70°F did not show any appreciable loss. Technical methoprene is soluble in water at 1.39 ppm and very soluble in all common organic solvents. The specific gravity of technical methoprene is 0.9261 at 20°C.

Technical methoprene is intended only for reformulation into an end-use pesticide, and therefore is considered a "manufacturing-use" product.

Topical Discussions

Chemical Identity

The Agency requires identifying information including chemical names, product names, and numerical codes of all substances known or assumed to be present in pesticide products.

The chemical name of methoprene is isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate or isopropyl-11-methoxy-3,7,11-trimethyl-2,4,-dodecadienoate. Trade and other names for methoprene include Altosid, ZR-515, and ENT-70460 (Zoecon, 1973?, 00012800; Zoecon, 1972, 00011495; Zoecon, 1973, 00011482). The empirical formula is $C_{19}H_{34}O_3$ and the structural formula (Zoecon, 1973, 00011483) is:

The Chemical Abstracts Registry number for methoprene is 40596-69-8 and the EPA Shaughnessy number is 105401. The common name, methoprene, will be used throughout this Standard in lieu of other chemical or trade names.

Manufacturing Processes

The chemical reactions employed in the synthesis and/or purification of the active ingredient may suggest the presence of potential harmful impurities and can serve as a verification of product composition. Discussions of specific procedures, equipment, and conditions concerning the commercial manufacture of methoprene are considered confidential business information and cannot be published in the body of the Standard.

Data on the manufacturing processes of methoprene have been provided by Zoecon Corporation and are discussed in the Confidential Appendix to this Standard. These data satisfy Agency requirements on manufacturing processes (Zoecon, 19??, 00010930; Zoecon, 1974?, 00010656; Zoecon, 1974?, 00010482).

According to the Farm Chemical Handbook, 1981, Zoecon Corporation, Palo Alto, California, is the only producer of methoprene in the United States.

No data on the manufacturing process of methoprene are available in the open literature.

Formation of Unintentional Ingredients

Detailed information on unintentional impurities which may be present in technical methoprene as produced by Zoecon Corporation has been submitted and is adequate (Zoecon, 197?, 00010973; Zoecon, 197?, 00010808). This information is considered confidential business information and is presented in the Confidential Appendix to this Standard.

Active Ingredient Limits in Pesticide Products

A statement of formula submitted by Zoecon Corporation stated that its technical methoprene contains about 92% of isopropyl-(2E, 4E)-11-methoxy-3,7,11-trimethyl-2,4,-dodecadienoate as the active ingredient (Zoecon, 1973, 00011482). The remaining percentage consists of manufacturing impurities which are detailed in the Confidential Appendix to this Standard.

The percentages of methoprene in currently registered products are:

Manufacturing-use Products	
Kabat Tobacco Protector Concentrate	80%
RF-179 Methoprene Concentrate	15%
End-use Products	
Altosid SR-10	10%
Altosid CP-10	10%
Starbar Block IGR	0.01%
Cattle Supplement Granular IGR	0.02%
Cattle Supplement Block IGR Cattle Supplement	0.0 5%
Altosid Briquets	7.9%
Kabat Tobacco Protector	5.0%
Kabat MC-6	80%
RF-174 Flea Growth Regulator with Precor	65.7%
RF-178 Fogger with Precor	0.15%
Moorman's IGR Minerals	0.02%
Moorman's IGR Mineral Block	0.02%
Moorman's Special Phos IGR Minerals	0.02%
Hi-Mag IGR Minerals	0.02%

The Confidential Statements of Formula have been examined for the formulations listed above. All of the inert ingredients in these formulations are cleared under 40 CFR 180.1001.

Product Analytical Methods and Data

Analytical methods for the identification and quantification of methoprene in the technical material and formulated end-use products have been submitted (Dunham, 1978, 05013295, Zoecon, 19??, 00010699; Moorman, 1975, 00010621; Rhoades, 1977, 00010907; McFall-Quistad, 1975, 00010883; Lowe, 19??, 00010665; Thalheimer, 1979, 00010925; Nicoara, 1976, 00011103; Zoecon, 19??, 00010971; Wenik, 1973, 00010806). The methods have been developed specifically for each particular end-use formulation.

Overall these methods are derived through small modification from one basic procedure which extracts the methoprene by a suitable solvent from the formulation. After additional clean-up by liquid partitioning the recovered methoprene is determined by gas-liquid chromatography. The column consists of a 2M x 3 mm (i.d.) column packed with 3% w/w methyl silicone (OV-101) on 100/120 Chromasorb W-AW-DMCS. The gas-chromatograph is equipped with a flame ionization detector.

Methods for the analysis of unintentional impurities have also been submitted and are considered to be adequate (Wenik, 1976, 00010697; Zoecon, 19??, 00010808). The methods consist of gas-liquid chromatographic and mass spectrometric analysis. Additional precision was obtained by the use of high pressure liquid chromatography using an ultraviolet photometer as a detector.

Physical and Chemical Properties

For every pesticide product, the Agency requires data on certain physical and chemical properties useful for identification purposes or for evaluation of hazard potential.

The physical and chemical properties for technical methoprene have been reported (Zoecon, 1972, 00010927) and are presented below:

Color	Pale yellow
Odor	Faint fruity odor
Boiling point	100°C at 0.05 mm Hg
Solubility	All common organic solvents; water at 1.39 ppm
Stability	Samples stored in glass for 4 years at 70°F did not show any appreciable loss. Other samples of methoprene stored for 4 years at 110°F did not show any loss in content.

Physical State	Liquid
Specific Gravity	0.9261 at 20°C
Vapor Pressue	2.37 x 10^{-5} mm Hg at 25°C 1.60 x 10^{-4} mm Hg at 40°C
pH	No pH is required since methoprene is practically insoluble in water.

Information on the physical and chemical properties of manufacturing-use products is also required. Since technical methoprene is a manufacturing-use product, information on physical state, specific gravity, and pH is the same as that presented above for technical methoprene. The following presents information for methoprene manufacturing-use products (Zoecon, 1972, 00010927; Dunham, 1978, 05013295):

Storage Stability	Methoprene is temperature stable up to at least 42°C for 24 months.
Flammability	370°F (COC method)
Oxidizing or Reducing Action	Methoprene is stable in the presence of dilute alkali and acid. Therefore, under dilute alkaline and acid conditions there will be no significant degradation of methoprene.
Explosiveness	Not reported
Miscibility	Not reported
Viscosity	Not reported
Corrosion Characteristics	Tests with 5 different methods show methoprene to have a zero to slight corrosion potential.

Product Chemistry: End-use Formulations

The following physical/chemical properties data have been reported for most of the end-use formulations: color, odor, physical state, and storage stability. For most of the end-use products, flammability, oxidizing and reducing action, explosiveness, miscibility and viscosity are not reported and constitute a data gap depending on the inert ingredients contained in the product. All of the inert ingredients contained in the end-use products are cleared for food use under Section 180.1001 of the Code of Federal Regulations 40, Part I.

Chapter V

Environmental Fate

Use Profile

Methoprene is an insect growth regulator (larvicide) federally registered for use in feed/mineral supplements for cattle, rice, pastures, stored tobacco, mushroom cultures, in and around homes, and on non-crop aquatic areas to control the horn fly, mosquito, cigarette beetle, tobacco moth, sciarid fly and flea larvae.

Approximately 57% of all the active ingredient methoprene that is sold domestically is used for horn fly control. Mineral block and mineral pellet formulations of methoprene are used systemically to control horn fly larva on cattle. These formulations are fed to beef and dairy cattle on a daily basis. Such formulations are normally placed near highly frequented areas, such as ponds, lakes, and loafing areas, to make them more accessible to cattle in order to ensure that the recommended daily dosages are received. These dosages are 0.25-0.5 oz of 0.01% pelleted/tableted, 0.5-1.0 oz of 0.005% pelleted/tableted, or 4-8 oz of 0.02 granular pelleted/tableted per 100 pounds of body weight.

Emulsifiable concentrate and briquet formulations of methoprene are sold domestically for the control of floodwater mosquito larvae in pastures, rice and non-crop aquatic areas. This use represents approximately 35% of all the active ingredient sold domestically. The rates of application for this use range from 0.01-0.03 lb active ingredient per acre for the EC, and 0.001 lb active ingredient per 100 square feet or 0.001 lb active ingredient per 10 cubic feet for the briquet formulation.

The emulsifiable concentrate formulation is also used to a minor degree on stored tobacco, mushroom cultures, and in and around domestic dwellings. The rate of application of methoprene on stored tobacco is 0.01 lb active ingredient per 1000 pounds. It is applied to tobacco leaves prior to placement in storage. The major use areas are North Carolina, Kentucky, and Virginia.

Methoprene is used to control mealy bugs and spider mites on houseplants. For this use a concentrated and diluted form is sold and applied with a hand-held pump sprayer. A small percentage (1-2%) of the active ingredient is sold annually for this use, primarily in the southern and western areas of the U.S.

Environmental Fate Profile

Aqueous solutions of methoprene are not hydrolyzed at pH 5, 7, or 9 at 20°C for up to 30 days, indicating that hydrolysis will not be a significant route of methoprene degradation under normal environmental conditions.

Methoprene is rapidly photodegraded in aqueous solutions exposed to natural sunlight. Complete degradation occurs within 7 days, resulting in the formation of at least 50 minor photolysis products (all 10% of applied).

These include methoxycitronellal, methoxycitronellic acid, (2E)-4-5-epoxy-11-methoxy-3,7,11-trimethyl-2-dodecenoate, and 8-methoxy-4,8-dimethyl-2-nonanone. Photolysis of aqueous solutions of methoprene occurs more rapidly at 40°C than at 24°C. Photoisomerization of trans-2-methoprene to cis-2-methoprene (biologically inactive) rapidly (within 9 hours) results in equimolar ratios of these two isomers.

Methoprene is rapidly photodegraded on inert surfaces, forming methoxycitronellal.

Methoprene is rapidly metabolized in soil under both aerobic and anaerobic conditions (half-life 10-14 days). The major product is CO_2, but 54% of the applied methoprene was bound to soil within 60 days. The rate of methoprene metabolism decreases as the application rate increases.

Methoprene is rapidly degraded in both sterile and nonsterile pond water exposed to sunlight. 7-Methoxy-3,7-dimethyloctanoic acid was the major degradation product under nonsterile conditions but was not formed under sterile conditions. Degradation is somewhat less rapid under sterile conditions than under nonsterile conditions, indicating that, although photolysis may be the main degradation route, microbial metabolism contributes to methoprene degradation.

Methoprene metabolism occurs at approximately the same rate in both freshwater and saltwater maintained in the dark. Metabolism proceeds more rapidly at 20°C than at 4.5°C, with associated half-lives of 10-35 days and 35 days, respectively. An emulsifiable concentrate formulation of methoprene is metabolized more rapidly than a slow-release flowable liquid formulation.

Microbial metabolism appears to be the major route of methoprene degradation in soil since degradation is 4-10 times more rapid in nonsterile than in sterile soil.

Methoprene is not likely to leach since it is relatively immobile (leaches no deeper than 3 cm) in four soils. The rapid adsorption of methoprene to soil further indicates that methoprene has a low leaching potential.

The dissipation of methoprene from the aquatic environment is rapid. Over 90% of the initial methoprene, applied as emulsifiable concentrates, flowable concentrates, or briquets at 0.05-2.0 lb ai/A, dissipates from a variety of aquatic sites within 3 days after application.

Methoprene residues accumulate in edible tissues of bluegill sunfish and crayfish at maximum bioaccumulation factors of 457 and 75, respectively. The nature of the residues and their rates of depuration are unknown.

Topical Discussions

Degradation

Hydrolysis

Hydrolysis data are required to support the registration of each end-use product intended for outdoor use of aquatic impact use, and each manufacturing-use product that may legally be used to formulate such an end-use product.

In one study, data showed that [^{14}C] methoprene was stable at pH 5-9 at 20°C. No methoprene degradation had occurred after 30 days (end of experiment) at pH 7 and 9 or after 21 days at pH 5 (Schooley and Creswell, 1972, 00010439).

No additional testing is required.

Photodegradation in Water

A photodegradation study in water is required to support the registration of each end-use product intended for terrestrial (except greenhouse and domestic outdoor), aquatic, and forestry use and for any aquatic impact use which results in direct discharges into the aquatic environment. This study is also required to support the registration of each manufacturing-use product which legally could be used to make such an end-use product.

Available data show that [^{14}C] methoprene photodegrades in an aqueous solution within 7 days (Staiger et al., 1973, 00010542; Quistad et al., 1975, 05008610). Fifty minor photolysis products (all <10% of applied) were found. Among these products were: methoxycitronellal (9% of applied), methoxycitronellic acid (7% of applied), (2E)-4,5-epoxy-11-methoxy-3,7,11-trimethyl-2-dodecenoate (4% of applied), and 8-methoxy-4,8-dimethyl-2-nonanone (4% of applied). This study is not acceptable because of the lack of information about the type of water used, pH, and the L_{50} (half-life).

In other studies, data show that [^{3}H] methoprene in sterile distilled water at pH 6 at 24°C was stable to photolysis for 9 hours (Zoecon, 1972, 00010935; Zoecon, 1972?, 00010440). At 40°C, 5% of methoprene had been photodegraded, producing more polar products. In a buffered solution at pH 7 and 20°C, 33% of the methoprene was degraded in 3 days. Photoisomerization of trans -2-methoprene to cis-2-methoprene altered the trans-2:cis-2 ratio from 97:3 to approximately 1:1 in both distilled water and buffered solutions.

The above studies are not acceptable to fulfill the requirements for this topic. Testing is required using either distilled or deionized H_2O at a specified pH which will not cause hydrolysis itself. Details on the sampling intervals, half-life and phtolytic products must be provided.

Photodegradation in Soil

Photodegradation studies on soil surfaces are required to support the registration of each end-use product intended for orchard crop use, field and vegetable crop use, or forestry use. Such studies are also required to support the registration of each manufacturing-use product which legally could be used to make such an end-use product. However, uses involving injection of the product into the soil or incorporation of the product into the soil upon application are not subject to the requirements of this section.

Data indicate that [^{14}C] methoprene is rapidly photodegraded (24% in 6 hours) on silica gel TLC plates (Staiger et al., 1973, 00010542; Quistad et al., 1975, 05008610). The only degradation product found was methoxyci-

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tronellal (14% of applied). When rose bengal and anthraquinone (photosensitizers) were added to the TLC plates, 56 and 86%, respectively, of the methoprene was degraded in 6 hours.

Additional testing is not required.

Photodegradation in Air

A laboratory photodegradation study in the vapor phase will be required on a case-by-case basis to support the registration of an end-use product with orchard or field and vegetable crop uses that involve potentially significant exposure to workers. Data from such a study will also be required to support the registration of a manufacturing-use product which legally could be used to make such an end-use product. The Agency will make an assessment of what constitutes a significant inhalation exposure to workers based on the information required by Section 163.163-2(b)(20).

No data are available on the photodegradation of methoprene in air. Data may be required, depending upon the results of the required volatility tests specified in Section 163.163-2, 163.163-3.

Metabolism

Data on metabolism are required to determine the nature and availability of pesticide residues to rotational crops and to help in the assessment of potential disposal and reentry hazards.

Aerobic Soil

An aerobic laboratory soil metabolism study is required to support the registration of each end-use product intended for terrestrial or forestry use. Such a study is also required to support the registration of each manufacturing-use product which legally could be used to make such an end-use product.

Data indicate that [^{14}C] methoprene applied at 1 lb/A degrades rapidly in sandy loam soil with a half-life of approximately 10 days (Staiger and Quistad, 1977, 00010541; Schooley et al., 1975, 05008315; Zoecon, 1972, 00010420; Zoecon, 1972, 00010874). The major product was Co_2 (49% of applied at 30 days after treatment). At 60 days after treatment, soilbound residues accounted for 54% of the applied radioactivity. The degradation rate was slower (half-life 30 days) when methoprene was applied at 10 lb/A to sandy loam soil. The half-life of methoprene in silt loam soil was about 14 days.

The studies cited above are not acceptable for the following reasons: controls in silt loam were not used, controls on soil moisture were not used, no material balance was done, and the incubation was done under light. Additional testing is required.

Anaerobic Soil

An anaerobic soil metabolism study is required to support the registration of each end-use product intended for field or vegetable crop use. Such a study is also required to support the registration of each manufacturing-use product which legally could be used to make such an end-use product. However, an anerobic soil metabolism study shall not be required if an adequate anaerobic aquatic metabolism study has been conducted.

Data indicate that [^{14}C] methoprene, applied at 1 lb/A, rapidly degrades in anaerobic sandy loam soil with a half-life of about 10 days. Further testing is not required.

Anaerobic Aquatic

An anaerobic aquatic metabolism study is required to support the registration of each end-use product intended for aquatic use, forestry use, or for any aquatic impact use which results in direct discharge into the aquatic environment. Such a study is also required to support the registration of each manufacturing-use product which legally could be used to make such an end-use product.

No data are available on the anaerobic aquatic metabolism of methoprene. Tessting is needed.

Aerobic Aquatic

An aerobic aquatic metabolism study is required to support the registration of each end-use product intended for aquatic use or for any aquatic impact use which results in direct discharge into the aquatic environment. Such a study is also required to support the registration of each manufacturing-use product which legally could be used to make such an end-use product.

Available data indicate that [^{14}C] methoprene is rapidly degraded in sterile and nonsterile pond water (Schooley et al., 1975, 05008622; Zoecon, 19??, 00010442). In nonsterile water exposed to sunlight, all of the methoprene was degraded within 13 days. Approximately 48% of the applied radioactivity remained without sediment after 13 days, and the major degradation product was 7-methoxy-3,7-dimethyloctanoic acid (methoxycitronellic acid; 29% of applied radioactivity). In sterile water exposed to sunlight, 10-20% of the methoprene remained and 98% of the applied radioactivity was still present in the form of numerous unidentified products. No 7-Methoxy-dimethyloctanoic acid was found in the sterile water samples.

A mosquito bioassay showed that technical methoprene controlled the adult emergence of adult mosquitoes from larvae in sterile sewer water for an unspecified period of time but did not control adult emergence in nonsterile sewer water (Schaefer, 1971, 00010974; Schaefer, 1971, 00010975). These data show that methoprene is degraded under nonsterile conditions.

Pree and Stewart (1975, 05009396) found that methoprene (slow release flowable liquid) slowly degrades in freshwater (pH 5.5) and saltwater (pH 7.4) in the dark. At 20°C, the half-life of methoprene was 28-35 days with 2-15% of the methoprene applied remaining at 132 days after treatment. At 4.5°C, over 85% of the applied methoprene remained after 132 days. Degradation was slightly more rapid in freshwater. When methoprene emulsifiable concentrate was applied, the half-lives of methoprene at 4.5°C were 50 and 35 days in saltwater and freshwater, respectively. At 20°C, the half-life of methoprene emulsifiable concentrate was 10-15 days in both freshwater and saltwater. Methoprene persisted for more that 134 days at 4.5°C and for 40-60 days at 20°C. The degradation products of this study were not identified.

Based on these data, methoprene appears to be metabolized by aquatic microorganisms to 7-methoxy-3,7-dimethyloctanoic acid, but the major degradation pathway of methoprene appears to be through either hydrolysis or photolysis.

Microbiological

Effects of Microbes on Pesticides

Data indicate that [^{14}C] methoprene degrades 4-10 times faster in nonsterile sandy loam soil than in sterile sandy loam soil (Staiger and Quistad, 19??, 00010541; Schooley et al., 1975, 05008315; Zoecon, 1972, 00010420; Zoecon, 1972, 00010874).

The requirement for these data is reserved pending the development and modification of test protocols.

Effects of Pesticides on Microbes

No data are available on the effects of methoprene on microbes. The requirement for these data is reserved pending the development and modification of test protocols.

Activated Sludge

No data are available on the effects of methoprene on activated sludge systems. The requirement for these data is reserved pending the development and modification of test protocols.

Mobility

The movement of pesticide residues by means of leaching through the soil may cause contamination of food, result in a loss of usable land and water resources to man due to contamination of ground water supplies, or cause habitat loss to wildlife. Therefore, studies are required to ascertain the extent of pesticide leaching through soil, which in turn provide a basis for assessing the mobility potential of a pesticide.

Leaching

Data are required to support the registration of each end-use product intended for domestic outdoor use, greenhouse use, terrestrial noncrop use, orchard crop use, field or vegetable crop use, forestry use, aquatic use, and aquatic impact use involving direct discharge only. Such data are also required to support the registration of each manufacturing-use product which legally could be used to make such an end-use product.

For terrestrial noncrop uses, field or vegetable crop uses, and forestry uses, the mobility of the test substance and its degradation products in soil shall be assessed either by soil thin-layer chromatography, soil column, or batch equilibrium (adsorption/desorption). For domestic outdoor uses, greenhouse uses, aquatic uses, and aquatic impact uses, the mobility of the test substance and its degradates in soil shall be assessed only by batch equilibrium (adsorption/desorption).

Data indicate that methoprene (4E and SR polyamide encapsulate formulations), when applied to sand, sandy loam, silt loam, and clay loam soil columns at 1 lb. ai/A and eluted with the equivalent of 20 acre-inches of water, is not mobile (Zoecon, 1972?, 00010444). Between 48 and 95% of the methoprene applied was recovered and all of the methoprene recovered was in the top 3 cm of the soil columns.

In another study, [^{14}C] methoprene at 0101 ppm adsorped readily to a sandy loam soil (Staiger and Quistad, 1973?, 00010507).

The studies cited above are not acceptable for these reasons: the substance was not aged in the soil prior to the test, the batch equilibrium process was not followed, and there were inconsistencies in the reporting of the data. Additional testing is required.

Laboratory Volatility

A laboratory volatility study will be required on a case-by-case basis to support the registration of each end-use product intended for commercial greenhouse, orchard, or field and vegetable crop uses that involve significant inhalation exposure to workers. Data from such a study will also be required to support the registration of each manufacturing-use product which legally could be used to make any end-use product for which laboratory volatility data are required.

No data on the laboratory volatility of methoprene are available. Testing is required.

Field Volatility

A volatility study conducted on-site in a commercial greenhouse and/or in the field will be required on case-by-case basis only for those pesticides that the Agency considers pose a potentially significant inhalation exposure to workers and where there is a significant rate of volatilization from soil.

No data on the field volatility of methoprene are available. The requirement for those data is reserved pending the results and evaluation of the laboratory volatility tests.

Dissipation

Field Dissipation: Terrestrial

A terrestrial field dissipation study is required to support the registration of each end-use product intended for any terrestrial use (except greenhouse use). Such data are also required to support the registration of each manufacturing-use product which legally could be used to make such an end-use product.

No data on the terrestrial dissipation of methoprene are available. Testing is required.

Field Dissipation: Aquatic and Aquatic Impact

An aquatic field dissipation study is required to support the registration of each end-use product intended for aquatic food crop uses, aquatic non-crop uses, and for any aquatic impact use which results in direct discharge into the aquatic environment. Such a study is also required to support the registration of each manufacturing-use product which legally could be used to make such an end-use product.

A summary of the aquatic field dissipation data is presented in Table 1 of this chapter. These data indicate that methoprene dissipates rapidly in water. Over 90% of the applied methoprene within 3 days after treatment. Methoprene also appears to dissipate rapidly in soil from submerged fields, remaining at 0.002 ppm (1% of the zero day concentration) in the top 2 inches of soil 7 days after treatment.

No additional testing is required.

Dissipation: Forestry

Field dissipation studies for forestry uses are required to support the registration of each end-use product intended for forestry use and of each manufacturing-use product which legally could be used to make such an end-use product.

No data are available on the dissipation of methoprene in a forest environment. Testing is not required.

Dissipation: Combinations and Tank Mixes

A laboratory or field soil dissipation study may be required on a case-by-case basis to support the registration of an end-use product containing more than one active ingredient, intended for use as a component in tank mixtures, or customarily applied serially with another pesticide product.

No data on the dissipation of multiple active ingredient formulations of methoprene are available. Data requirements for combinations and tank mixes containing methoprene are not cited here because this standard deals only with the single active ingredient.

Dissipation: Long Term

A long-term soil dissipation study will be required to support the registration of the end-use products of any pesticide that has been shown not to readily dissipate in a soil environment. Such a study may also be required to support the registration of any manufacturing-use that legally could be used to make such an end-use product.

No data on the long-term field dissipation of methoprene are available. The requirement will be imposed if the dissipation rate is shown to be less than 50%.

Table 1. Methoprene dissipation from several aquatic sites.

Site[a]	Application rate (lb ai/A)	Methoprene Formulation	Water Concentration on the day of application (ppm)	Postapplication concentrations (ppm)		Detection limit (ppb)
				In soil days after treatment)	In water (days after treatment)	
1	0.05-03	4 lb ai/gal emulsifiable concentrate, technical 70% flowable 10% flowable (slow release	0.05-1.04	—[b]	ND[c] (2)	0.3
2	0.5-2.0	4 lb ai/gal emulsifiable concentrate	0.12-0.81	—	ND (3)	10.0
3	0.1	10% flowable (slow release)	0.26	—	ND (3)	1.0
4	0.1-0.3	4 lb ai/gal emulsifiable concentrate	0.24-0.53	—	ND (2)	1.0
5	0.05-0.1	10% flowable (slow release)	0.015-0.09	ND (5)	ND (1)	1.0
6	0.05	10% flowable (slow release	—	0.028 (3) 0.002 (7)	—	1.0
7	2.0	10% flowable (slow release)	0.18	—	ND (6) $\leq$0.006 (7)[d]	1.0
8	0.38	briquet	ND	—	ND (up to 25)	0.4

[a] Sites were: 1, ponds, submerged pastures, duck habitats (Schaefer and Dupras, 05008625); 2, rice field in Baton Rouge, Louisiana (Steelman, 00011485); 3, marsh in Oroville, California (Hazeltine, 00012729); 4, ponds in Bakersfield, California (Schaefer, 00010434, 00010435, and 00010436); 5, submerged pasture field in Fresno, California (Schaefer, 00010437); 6, dry pasture field in Ajuba City, California, which was flooded after treatment (Kauffman, 00010438 and Zoecon Corp., 00011484); 7, sewage lagoon with drainage system in Baton Rouge, Louisiana (Steelman, 00010433 and Zoexon Corp., 00010417); 8, a ditch and a pond treated at four monthly intervals once, respectively, at 0.38 lb ai/A (Bledler, 00011091) and 00011092).

[b] Not reported.

[c] None detected.

[d] Samples from the lagoon drainage system 100-1000 feet from the lagoon.

Accumulation

Confined Accumulation: Rotational Crops

Confined acumulation studies on rotational crops are required to support the registration of each end-use product intended for field or vegetable crop use, aquatic crop use, or use on any other site on which it is reasonably foreseeable that any food or feed crop may be produced after application of a pesticide. Such studies are also required to support the registration of each manufacturing-use poduct which legally could be used to make such an end-use product.

No data on the confined accumulation of methoprene in rotational crops are available. Testing is required.

Field Accumulation: Rotational Crops

A field accumulation study to determine the uptake of soil residues by rotational crops is required when (1) the confined accumulation study identifies the ^{14}C resideus in the crop as either parent compound, closely-related degradates, metabolites, and/or their conjugates or (2) a subsequent crop is treated with the same active ingredient as the initial crop.

No data on the field accumulation of methoprene in rotational crops are available. Testing will be required only if significant C^{14} residues are found or if a subsequent crop is treated with the same active ingredient as the initial crop:

Accumulation: Irrigated Crops

A study of residue accumulation in irrigated crops under actual field use conditions is required to support the registration of each end-use product intended for aquatic food crop or aquatic non-crop uses, for uses in and around holding ponds used for irrigation pruposes, or for uses that involve effluents and other discharges which in turn are used to irrigate crops. Such a study is also required to support the registration of each manufacturing-use product which legally could be used to make such an end-use product.

No data on the accumulation of methoprene in crops irrigated with methoprene-treated water are available. Testing is not required.

Laboratory Studies: Fish

A fish accumulation study is required to support the registration of each end-use product intended for outdoor use (except domestic outdoor and greenhouse uses), or aquatic impact use resulting in direct discharge into aquatic environments, and for each manufacturing-use product that legally could be used to produce such a product, except when the criteria below are satisfied.

Fish accumulation data will not normally be required in situations where the registrant can offer acceptable evidence showing that the active ingredient and/or its principal degradation product(s):

Will not reach water, or
Will not persist in water (i.e., a half-life of approximately 4 days or less) and has properties suggesting:
  - A relatively low potential for accumulation in fish (i.e., an octanol/water partition coefficient less than 1000) or
  - A lack of accumulation in the organs and tissues of mammals or birds.

Sleight (1973, 00012785) found that [^{14}C] methoprene residues accumulate in bluegill sunfish in a static system. [^{14}C]Methoprene was applied at 0.06 lb ai/A on days 1, 8, and 15 and ^{14}C residue levels in edible fish tissue reached a plateau within 7 days of each application. The ^{14}C concentration in the water dropped 80% (from 8.61 to 1.75 ppb), and the ^{14}C concentration in the soil increased 400% (from 3.8 to 19.0 ppb) during the first 7 days after treatment. On day 21, the maximum ^{14}C level in edible tissue was 2.78 ppm corresponding to a bioaccumulation factor of 457. These data are summarized in Table 2 of this chapter. ^{14}C remained at 1.67 ppm (bioaccumulation factor of 253) in the edible tissue at the end of the 42-day experiment.

Fish Accumulation: Aquatic Non-Target

Field accumulation studies in aquatic non-target organisms are required to support the registration of each end-use product:

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- Which is intended for forestry use, aquatic non-crop use, or aquatic impact use that results in direct discharge;
- For which data from a laboratory fish accumulation study are required by Section 163.165-4; and
- For which no tolerance or action level for fish has been granted.

Such studies are also required to support the registration of each manufacturing-use product which legally could be used to make such an end-use product.

In one study, using a static ecosystem containing soil treated with [^{14}C]methoprene and anaerobically aged for 30 days, it was determined that methoprene accumulated in crayfish (Sleight, 1975?, 00010858). The data from this study are presented in Table 3 of this chapter. The highest ^{14}C levels in the crayfish were 77 ppb on day 21 of the exposure period, corresponding to a bioaccumulation factor of 75. The bioaccumulation factor had declined to 60 at the end of the 42-day experiment. The ^{14}C concentration in the soil and water remained amost constant throughout the experiment.

Formulations of Methoprene

Methoprene is formulated into the following: 10% and 15% a.i. formulation intermediate; 0.02% a.i. granular; 0.005, 0.01, 0.02, and 7.9% a.i. pelleted/tableted; 0.34 and 0.86 lb a.i./gal emulsifiable concentrate. Of the amount of methoprene used annually, about 57% is used for feed-through horn fly control in cattle and 39% is used for mosquito control in agricultural, non-crop, and domestic aquatic sites. Minor uses include tobacco treatment and houseplant insect control. Methoprene is applied using aircraft and ground equipment or by hand. Applicators do not need to be certified to apply methoprene.

Data are not available to fully assess the potential for exposure of humans or wildlife to methoprene. The risks associated with any possible exposure appear to be small due to the low mammalian toxicity and low application rates. The rapid photodegradation of methoprene in water and rapid metabolism of methoprene in soil further lessen the opportunity for exposure.

Methoprene is rapidly and efficiently adsorbed to soil and does not leach deeper than 3 cm in a variety of soils. The negligible soil mobility indicates that methoprene should not contaminate ground water, and thus exposure via this route is unlikely.

Low to moderate accumulation of methoprene residues occurs in the edible tissues of bluegill sunfish and crayfish. Maximum bioaccumulation factors of 457 and 75, respectively, were recorded for these species. Additional information is required because the residues were not characterized and the rates of their depuration are unknown.

Human exposure due to inhalation of dislodgeable plant residues or during the mixing and application of methoprene cannot be assessed due to the lack of data. Respiratory exposure could occur during the application of liquid methoprene formulations to stored tobacco in an enclosed area. Face masks would minimize such exposure. The use of protective clothing and goggles would minimize any dermal or ocular exposure associated with reentry tasks or during the application of granular or pelleted/tableted methoprene formulations.

Table 2. Mean [^{14}C]methoprene levels in the edible tissue of bluegill sunfish, soil, and water during a 42-day model aquatic ecxogystem experiment.

Day	[^{14}C]Residue levels: Water (ug/l)	Soil (ug/kg)	Edible fish tissue (mg/kg)	Bio-accumulation factor [a]
1[b]	8.61	3.8	0.53	62
3	4.01	11.8	0.26	65
7	1.75	19.0	0.33	189
8[c]	9.32	15.0	0.79	85
10	5.05	27.5	0.86	170
14	3.36	25.0	0.56	167
15[d]	10.29	37.0	2.06	200
17	7.12	88.0	2.70	379
21	6.08	32.0	2.78	457
28	6.60	63.0	2.69	408
35	5.59	20.0	2.03	363
42	6.60	55.0	1.67	253

[a] Calculated by the reviewer.

[b] First application.

[c] Second application.

[d] Third application.

From 00012785.

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Table 3. Mean ^{14}C residues in soil, water, and edible crayfish tissue samples in a model ecosystem containing [^{14}C]-methoprene-treated soil.[a]

Time interval [b]	[^{14}C]Residue levels Soil (ug/kg)	Water (ug/kg)	Crayfish (ug/kg)	Bio-accumulation factor [c]
1	68.9	0.92	4.1	4
3	60.4	1.27	22.7	18
7	58.9	1.53	6.0	4
10	44.3	0.68	6.3	9
14	60.3	0.93	70.0	75
21	69.9	1.02	77.0	75
28	80.7	1.40	52.0	37
35	66.7	0.82	54.0	66
2	72.4	1.06	64.0	60
X	64.7	0.96	63.4[d]	66[d]

[a] Soil was aged for 30 days after being treated with [^{14}C]methoprene.
[b] Days after introduction of crayfish.
[c] Calculated by the reviewer.
[d] Mean calculated for the 14- to 42-day period.

From 00010858.

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Chapter VI

Toxicology

Toxicology Profile: Manufacturing-Use Methoprene

Acute Toxicity

Sufficient data are available to adequately assess the acute toxicity of technical methoprene. The acute oral LD_{50} value is determined to be greater than 10,000 mg/kg in rats and between 5,000 mg/kg and 10,000 mg/kg in dogs. The acute dermal LD_{50} in rabbits is determined to be greater than 2000 mg/kg, and the acute inhalation LC_{50} in rats is greater than 20 mg/L. These data indicate a low human acute toxicity potential for methoprene. Sufficient data are available to assess the primary eye and dermal irritation potentials of technical methoprene. In an eye irritation study, 0.1 ml of technical methoprene did not cause eye irritation or corneal opacity in rabbit eyes. Additionally, when rabbits were given 24-hour dermal exposure to 0.5 ml technical methoprene on shaved, abraded and unabraded skin, no dermal irritation was obsetved for up to 72 hours. These data indicate that methoprene is not a primary eye or dermal irritant.

Several skin sensitization tests are available for technical methoprene. A skin sensitizing test was conducted in guniea pigs. Undiluted methoprene was administered by multiple intradermal injection. A positive reaction was obtained. However, when the test was subsequently repeated by topical application the results were negative. Additionally, a test was performed according to a standard Draize method on human subjects to determine the human contact sensitization potential of the test chemical. No positive responses were observed and the test chemical is not a human skin sensitizer under the test conditions.

Data are not available to assess the acute toxicity of the manufacturing-use formulations containing 10-80% methoprene. Based on an evaluation of the inert ingredients, acute oral toxicity, acute dermal toxicity, and acute inhalation toxicity testing is required with the existing manufacturing-use product containing 15% methoprene.

No data are available to assess the primary eye irritation potential of the manufacturing-use products of methoprene. Based on an evaluation of the inert ingredients, eye irritation testing with each of these products is required.

Data are not available to adequately assess the primary dermal irritation and dermal sensitization potentials of the manufacturing-use methoprene. Based on an evaluation of the inert ingredients, these tests are required with the existing manufacturing-use product containing 15% methoprene.

Subchronic Toxicity

Data are available to assess the subchronic oral toxicity of methoprene in rats. In a 90-day feeding study, the no effect level (NOEL) for systemic toxicity was found to be 500 ppm. The lowest effect level was 1000 ppm. In dogs, a 90-day NOEL for systemic toxicity was established as 500 ppm and the lowest effect level was 5000 ppm. Considering the food conversion factor, these data indicate that the dog is more sensitive (NOEL = 12.5 mg/kg) to methoprene than the rat (NOEL = 25 mg/kg).

Data are available to adequately assess the subchronic dermal toxicity of methoprene. A 21-day dermal toxicity study in rabbits was conducted and satisfies the requirement for subchronic dermal toxicity testing. Five groups of five male and five female Japanese rabbits were acclimated for 20 days and treated with undiluted Altosid at 0, 100, 300, 900, or 2700 mg/kg/day for 30 days. The chemical was applied topically on the backs of the animals which were previously shaved. Except for redness observed from day 4 to 29 that the application sites in the highest dose group, no treatment related effects were observed. The NOEL is considered to be 100 mg/kg of body weight. Higher doses caused a decrease in body weight gain.

Data are available to assess the subchronic inhalation toxicity of technical methoprene. In a 21-day inhalation study in rats the NOEL was determined to be 20 mg/L (highest dose tested). Additional testing is not required.

Chronic Toxicity

Data are available to assess the chronic feeding toxicity and oncogenic potential of methoprene. In a 2-year feeding study with rats, the NOEL for systemic toxicity and oncogenicity was determined to be 5000 ppm (highest dose tested). In an 18-month oncogenicity study with mice, the NOEL for oncogenicity was determined to be 2500 ppm (highest dose tested), while the NOEL for systemic toxicity was established as 250 ppm.

Data are available to show that methoprene is not teratogenic or fetotoxic in rabbits at doses as high as 200 mg/kg, when administered on gestation days 6 through 15. At 2000 mg/kg (lowest effect level) maternal toxicity was observed in mice given 0, 50, 200 or 600 mg/kg/day methoprene on days 7 through 14 of gestation. A second teratogenicity study was conducted using mice. The data from this study support the conclusion that this chemical is not teratogenic in mice under test conditions. The NOEL is considered to be 600 mg/kg/day.

Data are available to assess the reproductive toxicity of methoprene in rats. In a three generation reproduction study, the NOEL was determined to be 2500 ppm (highest dose tested). Further testing is not required.

A bacterial assay and a dominant lethal study presented sufficient evidence to indicate that methoprene is not mutagenic. Although these studies are not adequate to fulfill mutagenicity data requirements, they do suggest a lack of genetic toxicity with methoprene. Since a mutagenic effect is not anticipated, this data requirement is waived at this time. The Agency reserves the right to ask for additional mutagenicity testing if a need is indicated in the future.

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A series of studies investigating the metabolism of methoprene in the rat, mouse, guinea pig, steer, and cow are available. These studies indicate rapid and extensive biodegradation of methoprene and its metabolites in mammalian species and that methoprene metabolites are incorporated into natural body constituents.

Data to assess the toxicity of methoprene in domestic animals are also available. In an 8-day feeding study in chicks, a NOEL of 4640 ppm (highest dose tested) was established. Based on food consumption and body weight data, an acute oral LD_{50} for methoprene in chicks was estimated to be greater than 1 g/kg. In a six-week feeding study in laying hens, 30 ppm (highest dose tested) was determined to be the NOEL. Methoprene at this concentration had no effect on egg production, feed intake, feed consumption, egg weight, shell thickness, albumin weight, Haugh Units, yolk color or hen weight.

Topical Discussions: Manufacturing-use Methoprene

Acute Toxicity

Acute Oral Toxicity

Adequate data are available to evaluate the acute oral toxicity of technical methoprene. Rats given a single oral dose of 10,000 mg/kg methoprene (68.9% A.I.) did not exhibit any signs of toxicity (Hallesy, 1972, 00024607). Body weight gain and food and water consumption were normal. At necropsy, no treatment-related gross pathological alterations were observed. The data indicate an acute oral LD_{50} of greater than 10,000 mg/kg for methoprene in rats.

In another study, 5 groups of 2 male Sprague-Dawley rats were given single oral doses of 10,200 to 51,900 mg/kg methoprene (68.9% A.I.) (Jorgenson, 1972, 00024613). One of the rats given 51,900 mg/kg died on the sixth day following treatment. No other mortalities were observed during the two-week observation period. Gross pathological examination showed blood colored fluid in the stomach and urinary bladder of the rat that died during the study. No gross pathological changes were observed in the surviving animals. This study also supports an acute oral LD_{50} of 10,000 mg/kg (rat) for technical methoprene.

Hallesy et al. (1972, 00024609) gave 2 male and 2 female beagle dogs a single oral dose of 10,000 mg/kg methoprene (68.9% A.I.) and observed signs of toxicity that included hyperactivity, dialated pupils, rapid respiration, ataxia, salivation, vomiting, and convulsions. Two hours after the treatment, three dogs had died and the fourth dog was sacrificed after 3 hours because of severe toxicity. Gross pathological examination showed congestion of the kidneys, liver, lungs, and scleral vessels and telangiectasis in the livers.

The above study was repeated using lower doses of methoprene. In this second study, three groups of one male and one female beagle dog were given single oral doses of 1000, 2000, or 5000 mg/kg methoprene (68.9% A.I.) and no deaths or clinical signs of toxicity were observed during a 21-day observation period. Body weight gain and food and water consumption were normal. At necropsy, no treatment-related pathological changes were observed. Based on these findings, an LD_{50} of greater than 5000 mg/kg but less than 10,000 mg/kg methoprene is indicated in dogs.

All of the available acute oral toxicity data indicate a low acute oral toxicity protential and place technical methoprene in Toxicity Category IV. No additional testing on the technical product is required.

No data are available to assess the acute oral toxicity of manufacturing-use methoprene products containing 10-80% methoprene. Based on an evaluation of the inert ingredients, an acute oral toxicity test is required on the manufacturing-use product containing 15% methoprene.

Data are available on the two primary metabolites of methoprene, ZR-724 and ZR-725. In a study, 4 groups of 5 male and 5 female Charles River CD rats were given a single oral dose of 2150, 3160, 4640, or 6810 mg/kg ZR-724 in PEG 300 solution (Johnson and Knott, 1973, GS0030-0004). One male and two females at the 4640 mg/kg dose and one male at the 6810 mg/kg dose died during the 14-day observation period. Other signs of toxicity included depression (at all doses) and salivation at the two highest doses. At necropsy, the male rat in the 4640 mg/kg dose group that died during the study showed weight loss and a bloated stomach. No other pathological observations were observed. Thus, the acute oral LD_{50} for ZR-724 in rats was determined to be greater than 6810 mg/kg.

In another study, 4 groups of 5 male and 5 female Charles River CD rats were given a single oral dose of 2150, 3160, 4640 or 6810 mg/kg ZR-725 (Johnston and Knott, 1972, GS0030-0004). Signs of toxicity included death of some rats at the three highest doses; salivation, depression and convulsions at 4640 and 6810 mg/kg. At the lower doses only depression was observed. At necropsy, one male, at 3160 mg/kg, that died during the study had a darkened liver and bloated stomach. In addition, one female at 4640 mg/kg, that died early, had an intestinal blockage. No other pathological alterations were observed. The acute oral LD_{50} for ZR-725 technical was determined to be 4870 mg/kg in female rats and greater than 6810 mg/kg in male rats.

Acute Dermal Toxicity

An acute dermal toxicity study in rabbits, using technical methoprene (68.9% A.I.), was conducted at Industrial Biotest Laboratories. The toxicological evaluation of this study is pending receipt of the results from the audit validation program.

In an adequate study, an emulsifiable concentrate formulation of methoprene (65.9% A.I.) did not produce mortality in rabbits after a 24-hour dermal exposure to a 2000 mg/kg dose of this product (Hepler et al., 1979, 00010914). The acute dermal LD_{50} for this product was greater than 2000 mg/kg and indicates a low potential for acute dermal toxicity. These data are considered adequate to fulfill acute dermal toxicity testing requirements for technical methoprene and place it in Toxicity Category III for acute dermal toxicity. No additional testing on the technical is required.

No data are available to assess the acute dermal toxicity potential of the manufacturing-use methoprene formulations containing 10-80% A.I. Based on an evaluation of the inert ingredients, an acute dermal toxicity test is required on the existing manufacturing-use product containing 15% methoprene.

Acute Inhalation Toxicity

The vapor pressure of technical methoprene is 2.37 x 10^{-5} mm Hg 25°C and 1.60 x 10^{-4} mm Hg at 40°C and indicates that methoprene is likely to form a respirable vapor. In addition, emulsifiable concentrate formulations of methoprene are applied by methods that would permit inhalation exposure. Therefore, acute inhalation testing is required.

Adequate data are available to assess the acute inhalation toxicity of technical methoprene. In one study, rats were exposed to 0, 2, or 20 mg/L of aerosolized methoprene (68.9% A.I.) for 4 hours a day, 5 days a week for 3 weeks (Olson and Willigan, 1972, GS0030-0009). No treatment-related mortalities, clinical signs of toxicity (other than nasal discharge at 20 mg/L) or pathological alterations were observed. An acute inhalation LC_{50} of greater than 29 mg/L is estimated. Based on these results, technical methoprene is placed in Toxicity Category IV for acute inhalation toxicity. No additional testing is required on technical methoprene.

No data are available to assess the acute inhalation toxicity of the manufacturing-use methoprene formulations containing 10-80% A.I. Based on an evaluation of the inert ingredients, an acute inhalation toxicity test is required with the manufacturing-use product containing 15% methoprene.

Primary Eye Irritation

Data are available to assess the primary eye irritation potential of technical methoprene. No eye irritation or corneal opacity was observed in rabbit eyes at 24, 48 and 72 hours following instillation of 0.1 ml of methoprene (68.9% A.I.) (Hallesy and Hill, 1972, 00024614). In addition, no clinical signs of toxicity were observed during a 3-day observation period. The primary eye irritation score was zero out of a possible 110. The test material is not a primary eye irritant and is placed in Toxicity Category IV for eye irritation. No additional testing is needed for technical methoprene.

No data are available to evaluate the primary irritation potential of the manufacturing-use formulations containing 10-30% methoprene. Based on an evaluation of inert ingredients, primary eye irritation testing with each existing product is required.

Primary Dermal Irritation

Adequate data are available to assess the primary dermal irritation potential of technical methoprene. Female New Zealand albino rabbits were exposed for 24 hours to 0.5 ml of methoprene (68.9%) (Hallesy and Hill, 1972, 00024615). The test material was applied to the shaved, abraded and unabraded, skin of each rabbit under an occlusive wrap. No dermal irritation was observed at 24 or 72 hours. The primary dermal irritation score was zero out of a possible 8. The test material is not a primary skin irritant, and is placed in Toxicity Category IV for dermal irritation. No additional testing is required.

No data are available to evaluate the primary dermal irritation potential of the manufacturing-use formulations containing 10-80% methoprene. Based on an evaluation of the inert ingredients, a primary dermal irritation test is required on the manufacturing-use product containing 15% methoprene.

Dermal Sensitization

Data are available to assess the dermal sensitization potential of methoprene. In one study, a total of 12 guinea pigs were used. Eight animals were treated with the test material (a white liquid identified as "House Plant Spray" by Zoecon) and the other four were used as positive controls. The positive control (0.1% 2,4-dinitro-1-chlorobenzene) or test compound was injected intradermally into a prepared area on the backs and flanks of the respective guinea pigs. The injection was made 3 times a week for a total of 10 injections. The test chemical was injected undiluted in a volume of 0.1 ml per injection. A new site was used for each of the 10 injections. Sodium chloride solution (0.9%) was used as a negative control. The injection sites were read and scored for diameter and intensity of erythema and height of edema at 24 and 48 hours after each injection. Two weeks following the tenth sensitizing dose, a challenge dose at a volume of 0.1 ml was given by intradermal injection of either the test compound or the positive control. Reaction to the challenge dose was read and scored at 24 and 48 hours following the unjection. All of the animals appeared normal and exhibited normal body weight gains throughout the study. All of the eight guinea pigs treated with the test chemical responded to the challenge dose, in both flare and wheal reactions; and showed a flare response which was greater than the average response obtained during the sensitizing phase.

In another study, 15 female albino guinea pigs were acclimated for two weeks, their left flanks were shaved and 0.5 ml of the undiluted test chemical (YOUR BRAND Insect and Mite Houseplant Mist by Zoecon) was applied. The material was held in contact using a pad of nonwoven fabric and secured by an elastoplast bandage over the circumference of the animal's trunk. All covering was removed from the application site 24 hours later. The induction procedure including shaving was repeated on days 7 and 14 with application being made to the same site as on day 0. On day 28, the test material was applied to a freshly shaved area on the opposite flank. The patch was removed after 24 hours. Approximately 5 hours prior to the 48-hour scoring of response to the challenge dose, the test site was chemically depilated. The treatment sites were read and scored 24, 48 and 96 hours after the challenge. No positive or negative controls were included and no sensitizing reaction occurred in any animal.

A test was performed, according to a standard Draize method, on human subjects to determine the human contact sensitization potential of methoprene. The test was performed on 231 volunteers. The application was made for both induction and challenge using 0.5 ml of the undiluted material (YOUR BRAND Insect and Mite Houseplant Mist by Zoecon). The material was held in contact with the skin using unperforated pads from Johnson and Johnson. All induction applications were made to the same site, the right dorsolateral area of the back. Challenge application was made to the dorsolateral surface of the right upper arm. Each of the 10 induction patches were removed at 48 or 72 hours and the underlying skin was read and scored. It is concluded that the test chemical is not a human skin sensitizer.

In addition to the above described studies, Zoecon has submitted records of human exposure through May 1977. Approximately 750 man-hours of exposure were recorded. Formulation chemists have logged 400 man-hours of exposure to Insect and Mite Houseplant Mist. In the course of assessment of efficacy, it is estimated that Zoecon research entomologists and independent cooperators accumulated 350 man-hours of exposure. There are no records of any adverse reaction in exposed individuals. Also, because of the low order of toxicity of this formulation, no unusual precautionary measures have been taken in the course of handling or applying this material.

No data are available to evaluate the dermal sensitization potential of the manufacturing-use formulations containing 10-80% methoprene. Based on an evaluation of the inert ingredients, testing of the dermal sensitization potential of the manufacturing-use products containing 15% methoprene is required.

Acute Delayed Neurotoxicity

The minimum data requirement for acute delayed neurotoxicity is one test on the technical material in hens. An acute delayed neurotoxicity test is required if the active ingredient or any of its metabolites, degradation products, or impurities cause esterase depression or are structurally related to a substance that induces delayed neurotoxicity. There is no indication that suggests that methoprene or its metabolites cause esterase depression or are structurally related to know neurotoxins. Acute delayed neurotoxitiy testing is not required.

Subchronic Effects

Subchronic Oral Toxicity

In a range-finding study, 6 groups of 5 male and 5 female Sprague-Dawley rats were given 0, 1000, 5000, 10,000, 20,000 or 40,000 ppm methoprene (68.9% A.I.) in the diet for 2 weeks (Jorgenson and Sasmore, 1972, 00024610). Food consumption and body weight gain were markedly depressed at the two highest doses. These effects were attributed to palatibility problems with the test material. After treatment with methoprene was discontinued in the third week, food consumption in the 20,000 and 40,000 ppm groups returned to normal. Except for food rejection with resulting weight loss, at the highest doses, no other clinical signs of toxicity were reported. At necropsy, gross pathological examination of the rats at the 40,000 ppm dose level revealed no abnormalities. An effect level could not be established from these data because of palatability problems with the test material.

In another range-finding study, five groups of three male beagle dogs were fed 0, 1000, 5000, 10,000, or 20,000 ppm methoprene (68.9% A.I.) in the diet for two weeks (Jorgenson and Sasmore, 1972, 00024610). A dose-related reduction in body weight gain, at the two highest doses, was observed. Food consumption was depressed in animals at 10,000 and 20,000 ppm during the treatment period. However, when methoprene treatment was discontinued during the third week of testing, food consumption in thee animals returned to normal. At necropsy a dose-related increase in liver weights was observed. Dogs at 10,000 and 20,000 ppm also displayed morphological changes in the liver, however these changes were not described. The data indicate that the dog is less tolerant of high doses of methoprene than the rat. Based on the body weight and food consumption data, 5000 ppm is an estimated maximum tolerated dose (MTD) for a subchronic feeding study with dogs.

In a 90-day feeding study, 15 male and 15 female Sprague-Dawley rats were fed diets containing 0, 250, 500, 1000, or 5000 ppm methoprene (68.9% A.I.) (Jorgenson and Sasmore, 1972, 00024612). A significant increase in liver weights was observed in both males and females at the highest dose. In addition, kidney weights in males at 5000 ppm were significantly increased. Renal tubular degeneration was observed in 7 out of 15 males at 5000 ppm and 3 out of 15 males at 1000 ppm. Based on these findings, a no effect level (NOEL) of 500 ppm is established. No additional subchronic oral toxicity testing in rats is required.

A 90-day feeding study using dogs is available for methoprene (Jorgenson and Sasmore, 1972, 00024612). In this study, four male and four female beagle dogs were fed diets containing 0, 250, 500, or 5000 ppm methoprene (68.9% A.I.). The results showed that liver weights for males and females at 5000 ppm were significantly elevated. In addition, serum alkaline phosphatase (SAP) levels in males at 5000 ppm were elevated at the 4, 8, and 13-week testing intervals. Females at this dose had elevated SAP levels at the 8-week interval only. No other treatment-related effects were observed. Taking into consideration the food conversion factor, the NOEL for dogs is lower (12.5 mg/kg) than for rats (25 mg/kg). These data suggests that the dog is more sensitive to methoprene than the rat.

Subchronic 21-day Dermal Toxicity

Data are available to adequately assess the subchronic dermal toxicity of methoprene. A 21-day dermal toxicity study in rabbits was conducted and satisfies the requirement for subchronic dermal toxicity testing. Five groups of five male and five female Japanese rabbits were acclimated for 20 days and treated with undiluted Altosid at 0, 100, 300, 900 or 2700 mg/kg/day for thirty days. The chemical was applied topically on the backs of the animals which were previously shaved. Except for redness observed from day 4 to 29 at the application sites in the highest dose group, no treatment-related effects were observed. The NOEL is considered to be 100 mg/kg of body weight. Higher doses caused a decrease in body weight gain.

Subchronic 90-day Dermal Toxicity

A subchronic 90-day dermal toxicity test is not required because methoprene is not intentionally applied to skin, and the use is not expected to result in significant human dermal exposure.

Subchronic Inhalation Toxicity

In a 21-day inhalation study, three groups of 10 male and 10 female albino rats were given repeated inhalation exposure to a nominal concentration of 0, 2, or 20 mg/L of aerosolized methoprene (68.9%) (Olson and Willigan, 1972, GS0030-0009). The animals were exposed to the test material 4 hours a day, 5 days a week, for 3 weeks. No treatment-related mortalities were observed during the study. Rats exposed to 20 mg/L of the test material had nasal discharge during the exposure period; no other clinical signs of toxicity were observed. Body weight gain was comparable among the different groups and clinical biochemistry and hematology parameters did not indicate a consistent pattern of toxicity. In addition, lung, liver, and kidney did not show

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compound-related alterations. A NOEL of 20 mg/L, which was the highest dose tested, is established from these data.

No additional subchronic inhalation toxicity testing is required for technical methoprene.

Subchronic Neurotoxicity

A subchronic neurotoxicity test is required if a pesticide has shown positive results in the acute delayed neurotoxicity test or induced irreversible neurological toxicity in mammalian species. There is no evidence to suggest that methoprene is neurotoxic. Testing is not required.

Chronic Effects

Chronic Feeding

In a chronic feeding study, rats were fed diets containing 0, 250, 1000, or 5000 ppm methoprene (86.9% A.I.) for two years (Wazeter and Goldenthal, 1976, 00010739). No treatment-related effects on body weight, food consumption, behavior, hematology, blood chemistry, urinalysis and organ weights were observed. Ophthalmoscopic examinations did not indicate a treatment-related effect. At the end of the study, survival was approximately 50% in all groups. At necropsy no treatment-related gross pathological or histopathological lesions were observed. In addition, there was no increase in tumor incidence in treated groups. The NOEL from this study is indicated as 5000 ppm, which is the highest dose tested. These data are adequate to fulfill the chronic feeding testing requirement for methoprene. Further chronic feeding testing is not needed at this time. However, the Agency reserves the right to ask for additional data if a need is indicated in the future.

Oncogenicity

In a study to evaluate the oncogenic potential of methoprene, four groups of 50 male and 50 female Charles River CD rats were fed diets containing 0, 250, 1000, or 5000 ppm methoprene (86.9% A.I.) for two years (Wazeter and Goldenthal, 1975, 00010739). No indication of an oncogenic effect was observed even at the highest dose level.

In another study, four groups of 50 male and 50 female Charles River CD-1 mice were fed diets containing 0, 250, 1000, or 2500 ppm methoprene (86.9-87.5%) for 18 months (Wazeter and Goldenthal, 1975, 00010600). No difference was observed in the tumor incidence between the control and treated groups. No treatment-related effects on body weight, food consumption and general behavior were observed. At 18 months, survival was approximately 50% in all groups. At necropsy no treatment-related gross pathological changes were observed. However, histopathological examination indicated a high incidence of brown pigmentation of liver at 2500 ppm. This finding was also observed in some mice at 1000 ppm, but was absent at the 250 ppm level and in the control group. In addition, a higher incidence of liver necrosis was observed in the females of the 5000 ppm group when compared to the control group. This difference was not observed in male mice. Anyloidosis was observed in various

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tissues at all dose levels including the control group. The incidence of this finding was approximately twice as great in high dosed animals as in the controls.

These data are adequate to fulfill oncogenicity testing requirements for methoprene. Additional testing is not required.

Teratogenicity

In a study to assess the teratogenic potential of methoprene, four groups of 10 pregnant rabbits were given 0, 50, 200, or 2000 mg/kg methoprene (95.7% A.I.) orally in olive oil on days 7 through 18 of gesatation (Nomura Research Institute, 1975, GS00300-0006, GS0030-0007). No teratogenic effects were observed. Maternal toxicity as demonstrated by a 38.4% reduction in body weight gain and a 20% incidence of abortion and fetotoxicity was observed at 2000 mg/kg/day. Based on the doses tested, the lowest effect level for maternal toxicity and embryolethality in utero is 2000 mg/kg/day and the NOEL is 200 mg/kg/day. This study fulfills teratology testing requirements in one mammalian species.

In another teratology study, four groups of at least 30 pregnant mice were given 0, 50, 200, or 600 mg/kg/day methoprene (95.7%) in olive oil, orally, on days 7 through 14 of gestation. On day 18 of pregnancy, 20-23 mice in each group were sacrificed. Caesarians were performed and the effects on the fetuses were assessed. The remaining pregnant mice were allowed to whelp naturally and rear their litters for 3 or 7 weeks. No maternal toxicity, fetotoxicity or teratogenicity was observed at the doses tested. This study fulfills teratology testing requirements in a second mammalian species. No further testing is required.

Reproduction

Data were available to assess the reproductive effects of methoprene (Killen and Rapp, 1974, 00010571). In a three generation reproduction study, three groups of male and female Long Evan rats were fed diets containing 0, 500, or 2500 ppm methoprene (86.9%-87.5% A.I.). No compound related effects on body weight, food consumption, mating, fertility, gestation, litter size, viability and lactation were observed. Gross pathological examination of f_3 offspring did not indicate compound-related toxicity. The NOEL was determined to be 2500 ppm, the highest dose tested. Further reproduction toxicity testing with methoprene is not required.

Mutagenicity

Although the mutagenicity testing requirements are not final, refer to the Agency's Proposed Guidelines (FR 43, No. 163, Tuesday, August 22, 1978) for information concerning the types of studies the Agency is considering. The following studies are representative and are likely to be required:

1. A mammalian in vitro point mutation test.
2. A sensitive sub-mammalian point mutation test (bacteria, fungi, insect).
3. A primary DNA damage test (i.e., sister chromatid exchange or unscheduled DNA synthesis).

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4. A mammalian _in vitro_ or _in vivo_ cytogenetics test. If this test suggests a positive result, a dominant lethal or heritable translocation test may be required.

After results from these test systems and other toxicology disciplines have been considered, additional testing may be required to further characterize or quantify the potential genetic risks.

The mutagenic potential of methoprene was evaluated in the Salmonella reverse mutation assay. The assays were run in the presence of metabolic activation only. No evidence of mutagenicity was observed. However, the sensitivity of the assay cannot be adequately evaluated since no data were available for the positive controls and only a small range of low doses was evaluated.

In a dominant lethal study, methoprene was given intraperitoneally to male rats at 20, 200 or 2000 mg/kg as a single or repeated dose (2 or 5 daily doses). Negative controls and positive controls were included. Rats were mated for 8 weeks. No dominant lethal effects were observed. This study does not provide a reliable evaluation of the dominant lethal potential of methoprene since the number of animals evaluated at each treatment regimen was small (5 males and 10 females) and a low proportion of matings resulted in pregnancy (even in the negative control group).

Although the above data do not satisfy the Agency's mutagencity testing requirements, they do suggest a lack of genetic toxicity with methoprene. Since a mutagenic effect is not anticipated, this data requirement can be waived at this time. However, the Agency reserves the right to ask for additional mutagenicity testing if a need is indicated in the future.

Metabolism

The metabolism of methoprene has been investigated in the rat, mouse, guinea pig, cow and steer. Experimental data have shown that methoprene is extensively metabolized in each of these mammalian species; in addition, the data provide evidence that methoprene metabolites are incorporated into natural body constituents.

In a series of experiments, the excretion retention balance, biliary excretion, plasma kinetics and tissue distribution of rediolabelled methoprene and its metabolites were investigated in rats (Chasseaud et al., 1974, 00010866; Hawkins et al., 1977, 05007755). Twenty-five Ash Wistar rats of both sexes, were given single oral doses of 5-^{14}C-methoprene (98% A.I.) in 70% ethanol. In eight rats, the radioactivity of urine, feces and expired CO_2 was measured for 5 days. At the end of this period, 31% of the administered radiolabel was excreted in the urine. Expired air accounted for 25.5% of the radiolabel at 24 hours and 38.8% at 5 days. Tissue retention of the radiolabel accounted for 17.2%. From these data, the maximum excretion half-life for about 60% of the radiolabel was estimated as about 10 hours, and about 107 hours for an additional 15%.

In this same study, when rats with cannulated bile ducts were given radiolabelled methoprene, an average of 27.4% of the label was recovered in the bile in 48 hours. These data were used to estimate the gastrointestinal absorption of methoprene which was indicated as 74% of the administered oral dose.

In the plasma kinetic portion of the experiment, blood samples were taken from the tail veins of 6 rats for up to 5 days following dosing with 5-C^{14}-methoprene. The results showed that plasma radioactivity peaked at approximately 6 hours and declined slowly with a half-life of about 48 hours during the second to fifth day of dosing. The total amount of radioactivity in the plasma at 6 hours was about 1.63% of the administered dose.

The tissue distribution study showed the distribution of radioactivity in rats at 1, 3, 6, 12, 24, 72, 120 and 288 hours following oral dosing with 25 mg/kg of radiolabelled methoprene. In this portion of the study, the highest concentrations of radioactivity were initially observed in the liver, kidney, lungs (peaking at 6 hours) and fat (peaking at 12 hours). After 5 days most of the residual radioactivity was in the fat (8.5% of dose) and muscle (2.2% of dose). Whole body autoradiographs showed extensive distribution of radioactivity; high levels were observed after 6 hours in the stomach, liver, small intestines, brown fat, and significant levels were present in the kidneys, salivary and lachrymal glands, brain, thyroid, thymus, spleen, lungs, adrenals, myocardium and bone marrow. Of special interest were the high levels of radioactivity observed in the adrenal cortex at 5 and 12 days. The authors suggested that the high levels of radioactivity observed here may indicate the incorporation of methoprene metabolites into pathways of steroid biosynthesis.

In the above tests, unchanged methoprene was observed only in the feces and was present in small amounts. Twelve radiolabelled metabolites were identified in the urine. Most of these metabolites were considered to be carboxylic acids since they were converted to less polar compounds on treatment with diazomethane. In the bile, the major portion of the radioactivity was associated with a very polar material that was unaffected by enzyme treatment. These data confirm the extensive and relatively rapid metabolism of methoprene in the rat.

The metabolism of methoprene was investigated in mice (Zoecon, 1972, 00010425; Cline et al., 1972, 00010424). In these studies, methoprene (tritium labeled at the C-10 position, specific activity 2 Cui/mM dissolved in 35% ethanol) was administered intragastrically to 10 Swiss mice, 8 males and 2 females, at midgestation. Drug dosage was calculated on the basis of 7.7 mCui/g body weight and administered in 0.1 ml of ethanol. The radioactivity in the urine, feces and whole body was monitored for up to 4 days. The results showed that 68% of the radioactivity was eliminated in the urine and 14% in the feces by 4 days. A glucuronide conjugate of ZR-724 (11-hydroxy-3.7-11-trimethyl-dodeca-2,4-dienoic acid) was a major urinary metabolite. In the feces, unchanged methoprene and metabolites similar to some observed in urine samples were detected. However, fecal metabolites were not qualitatively identified.

Whole body autoradiographs indicated transient radiolabel in the stomach, liver, small intestines and large intestines up to 24 hours following dosing. By 43 hours there was negligible retention of the radiolabel in the body. Placental transfer of the radioactivity was not detected in the pregnant mice.

In a study that was designed to give a preliminary quantification and identification of methoprene and its metabolites in urine, feces, exhaled air, blood and tissues, one guinea pig was given a single oral dose of 49 mg/kg of ^{14}C-labelled methoprene (95-98% A.I.) (Chamberlain et al., 1973, 00010379). The distribution and elimination of the radioactivity was monitored for 24 hours following dosing. The results showed that 24.3% of the radiolabel was recovered in the urine, 9.1% in the feces, and 17.2% in the exhaled air by 24 hours. Blood contained 18.18 ug ^{14}C-equivalents per gram weight while muscle contained 14.3 ug equivalents per gram (or 3.31 ug per gram wet) and fat contained 10.95 ug equivalents per gram of tissue.

Chromatrographic analyses indicated that approximately 79% of the fecal radioactivitity was unchanged methoprene. In the urine a large proportion of the recovered radiolabel was glucuronic acid conjugates of ZR-724 (11-hydroxy-3,7,11-trimethyl-dodeca-2,4-dienoic acid), ZR-725 (11-methoxy-3,7,11-trimethyl-dodeca-2,4-dienoic acid) and ZR-669 (isopropyl 1, hydroxy-3,7,11-trimethyl-dodeca-2,4-dienoate). The remaining metabolites were mostly unidentified polar compounds. No intact methoprene was detected in the urine.

Data were available to demonstrate the exhaustive biodegradation of methoprene in the steer (Zoecon, 1973, 00011491; Staiger et al., 1973, 00010879; Chamberlain et al., 1973, 00010380; Quistad et al., 1975, 05008609). In a study, the radioactivity in urine, feces, blood tissues and samples of expired air was monitored for up to two weeks in a steer given a single oral dose of 2g ^{14}C-methoprene (96.9% A.I.). The total recovery of radioactivity was 78% of the administered dose. Urine contained 21.6% of the recovered radiolabel (with peak levels observed at 36 hours) and feces accounted for 38.3% (peak levels were observed at 48 hours). Samples of expired CO_2 accounted for 2.7% of the recovered radiolabel while blood and tissues accounted for 13.5%. Blood levels of radioactivity peaked at 72 hours and remained high until sacrifice at 2 weeks. Tissue radioactivity was highest in the gall bladder, followed by the liver, lung, kidney, spleen, heart.

In the urine, up to 14 different radiolabelled metabolites were identified. Most of these metabolites were more polar than methoprene or its primary metabolites (i.e., ZR-669, ZR-724, or ZR-725). Fecal extracts contained up to 7 radioactive metabolites of which the parent methoprene was the principal component (5-50% depending on sampling time). At 72 hours the following radioactive components were identified in the feces: 3.2% methoprene, 12% ZR-669, 4% ZR-725, 4% ZR-724, and less than 1% as ZR-1995. The remaining radioactivity was unidentified polar metabolites.

Analysis of the radioactive distribution in selected tissues showed no residues of methoprene or its known metabolites. However, significant amounts of label were identified in the form of cholesterol and, in the case of bile, in cholic and deoxycholic acids. Chemical degradation of labelled cholesterol and cholic acid showed ^{14}C incorporation into the steroid nucleus corresponding to the biosynthetic incorporation of ^{14}C-2-acetate.

In the above study, 22% of the administered radiolabelled was unaccounted for experimentally. However, the authors attributed the loss to the sampling techniques used to collect expired air. The explanation is consistent with the observations in the rat and guinea pig that indicated significant recovery of radioactivity in expired CO_2.

The fate of radiolabelled methoprene (5-^{14}C-methoprene, 95% A.I.) was investigated in a pregnant lactating Jersey cow for up to 7 days following the oral administration of 207.6 mg of 5-^{14}C-methoprene (Zoecon, 1974 00010681; Quistad et al., 1974, 00010626; Chamberlain et al., 1974, 00010683). In this study, the total radioactivity recovered during 7 days accounted for 94% of the applied dose. The following distribution was observed: 19.7% in the urine (peak recovery at 30 hours), 30.3% in feces (peak recovery at 50 hours), an estimated 16.4% in expired CO_2 (peak recovery at 24 hours), 7.6% in milk and 20% in tissues. In the tissues, the highest concentrations of radiolabel were detected in the gall bladder, liver, kidney, ovaries, and lungs. Recovery of radiolabel from the fetus accounted for approximately 1% of the original dose. Approximately 0.8% of the recovered radiolabel in the milk was unchanged methoprene. Most of the radioactivity in the milk was incorporated into lipids, lactose,and proteins. Radiolabelled acetate was isolated from a 48 hour blood sample and provided good evidence for label recyclying and incorporation of methoprene metabolites into natural body constituents.

These data are considered adequate to fulfill methoprene metabolism data requirements. The findings indicate rapid and extensive biodegradation of methoprene and its metabolites in mammalian species. Evidence was presented to show that methoprene metabolites are incorporated into natural body constituents.

Accident Reports

The files of the Pesticide Incident Monitoring System (PIMS) were searched for reports involving methoprene. The monitoring system contains information on reported human exposures to pesticides from 1966 to July, 1979. The files contained one incident involving accidental human exposure to methoprene. In this case, a 2 1/2 year old boy remained asymptomatic after methoprene from a pressurized container got into this mouth. The accident occurred in the home.

Zoecon Corporation (1973, 00010793) reported 40 incidents involving accidental methoprene exposure to male chemical workers. The incidents involved 26 exposures to technical methoprene and 14 exposures to a formulated product (10% E.C.). Exposure to the technical product was through dermal contact in 12 out of 14 cases and via the vapors in two instances. In all cases, no overt signs of toxicity were observed. Treatment in the majority of incidents consisted of washing the chemical off with soap and water. No medical treatment was indicated.

In addition, the Zoecon report cited two other incidents involving accidental exposure to methoprene. In the most serious incident, a male plant operator was coated with methoprene on the forehead, neck, forearms and hand following an explosion. After washing with soap and water, the victim did not experience any dermal or clinical signs of toxicity. In the other incident (under undescribed circumstances), no ill effects resulted from the extended dermal and inhalation exposure to technical methoprene in a male worker.

Toxicology Profile: Emulsifiable Concentrate Methoprene Products

Data are available indicating a low acute toxicity potential for the emulsifiable concentrate (E.C.) formulations of methoprene in humans. The acute oral LD_{50} in rats was greater than 5000 mg/kg for a 65.9% E.C. product and greater than 10,000 mg/kg for a 10% E.C. product. The acute dermal LD_{50} in rabbits was greater than 2000 mg/kg for a 65.9% E.C. product and greater than 9000 mg/kg for a 10% E.C. product.

No data are available on the acute inhalation toxicity of the E.C. formulations. However, an evaluation of the inert ingredients and available acute toxicity data indicate a low toxicity potential for these products and data are not required.

Available data indicate that E.C. formulations of methoprene are not primary dermal irritants. When rabbits were exposed to 0.5 ml of a 65.9% E.C. product, the primary dermal irritation score was "0" out of a possible 8 score. Additionally, based on the Confidential Statement of Formulation (CSF), the 5% E.C. product is not anticipated to be a primary dermal irritant and additional testing is not required.

Data are available to assess the eye irritation potential of E.C. products containing 65.9% and 10% methoprene. The data indicate that these products are not primary eye irritants. However, in humans, transient eye irritation could result from the accidental spillage of these products into the eye. No data are available to assess the primary eye irritation potential of the 5% E.C. product. Based on an evaluation of the inert ingredients in the 5% product, eye irritation testing is needed.

No studies were available on the skin sensitization potential of the E.C. products of methoprene. Based on the inert ingredients and available acute data, dermal sensitization is not anticipated with the products and testing is not needed.

Topical Discussions: Emulsifiable Concentrate Methoprene Products

Acute Oral Toxicity

Data are available to assess the acute oral toxicity of E.C. products containing 65.9% and 10% A.I. methoprene. In one study, five male and five female Wistar rats were given a single oral dose of 5000 mg/kg of the 65.9% E.C. product (Hepler et al., 1979, 00010913). No mortalities or clinical signs of toxicity were observed during a 14-day observation period. At necropsy, no treatment-related gross pathological abnormalities were observed. The data indicate an acute oral LD_{50} of greater than 5000 mg/kg for this product. Thus, this product should be placed in Toxicity Category IV for acute oral toxicity.

In another study, no mortalities or clinical signs of toxicity were observed when 5 groups of 5 male and 5 female Sprague-Dawley rats were given single oral doses of 0.625 to 10,000 mg/kg of an E.C. containing 10% A.I. methoprene (Hallesy et al., 1972, 00024608). At necropsy, no treatment-related gross pathological changes were observed. The data indicate an acute oral LD_{50} of greater than 10,000 mg/kg for this product. Based on these results this product should be placed in Toxicity Category IV for acute oral toxicity.

No additional testing is needed for existing E.C. methoprene products.

Acute Dermal Toxicity

Adequate data were available to assess the acute dermal toxicity of an E.C. containing 65.9% A.I. methoprene (Hepler et al., 1979, 00010914). In this study, 4 male and 4 female New Zealand white rabbits were given 24-hour dermal exposure to 2000 mg/kg of an E.C. containing 65.9% A.I. methoprene. The test material was applied to the shaved, abraded and intact skin of the animals under an occlusive wrap. No deaths were observed. Two out of 8 animals lost weight during this study; however, this was attributed to a respiratory disorder and not to the chemical. No other clinical signs of toxicity were observed during a 14-day observation period. In addition, no edema or erythema was observed at the test site. However, the treated skin of all the rabbits was dry and stiff; scab formation (which was observed in all animals) sloughed off by 14 days and revealed healthy skin beneath. At necropsy, 3 out of 8 rabbits had respiratory congestion; this effect was not considered treatment-related. The data indicate a dermal LD_{50} of greater than 2000 mg/kg for this product. Based on these results, this product should be placed in Toxicity Category III for acute dermal toxicity.

Adequate data were also available to assess the acute dermal toxicity of an E.C. containing 10% A.I. methoprene (Olsen, 1972, 00024618). In this study, 3 groups of 4 New Zealand white rabbits were given 24-hour dermal exposure to 1 3 or 9 g/kg of an E.C. formulation containing 10% A.I. methoprene. The test material was applied to the shaved, abraded or intact, skin of the rabbit under an occlusive rubber wrap. No mortalities were observed during the 14-day observation period. Slight or moderate erythema was observed at some test sites at 24 hours, and it persisted to a slight degree in some animals at 48 hours. Blanched skin was also observed at some abraded sites. In addition, slight skin desquamation was observed at abraded sites on days 2 through 4. Two rabbits at 3 g/kg and 1 rabbit at 9 g/kg had pale brown kidneys at necropsy. The dermal LD_{50} of this product was determined to be greater than 9 g/kg. Thus, this product is placed in Toxicity Category III for acute dermal toxicity.

No further acute dermal toxicity testing is required with the existing E.C. products containing methoprene as their single active ingredient.

Acute Inhalation Toxicity

No data were available to assess the acute inhalation of the E.C. formulations containing 5-65.9% methoprene. Based on an evaluation of the inert ingredients and the available acute data that indicates a low toxicity potential for these products, acute inhalation toxicity testing is not required. These products should be placed in Toxicity Category IV for acute inhalation toxicity, based on the results from testing of the technical chemical.

Primary Eye Irritation

Adequate data were available to assess the primary eye irritation potential of an E.C. containing 65.9% A.I. methoprene (Hepler et al., 1979, 00010915). In this study, slight conjuctival irritation was observed in washed and unwashed rabbit eyes up to 72 hours following the instillation of 0.1 ml of an E.C. containing 65.9% A.I. methoprene. By 7 days all of the treated eyes appeared normal. This product is not a primary eye irritant and should be placed in Toxicity Category III for eye irritation.

Adequate data were also available to assess the primary eye irritation potential of an E.C. containing 10% A.I. methoprene (Hallesy and Hill, 1972, GS0030-0001). In this study, slight conjunctival irritation was observed in washed and unwashed rabbit eyes at 1 hour following the instillation of 0.1 ml of an E.C. containing 10% a.i. methoprene. The irritation cleared by 24 hours and the animals' eyes remained normal during the remaining 7 day observation period. This product is not a primary eye irritant and should be placed in Toxicity Category III for eye irritation.

No data were available to assess the primary eye irritation potential of an E.C. containing 5% methoprene. Based on an evaluation of the inert ingredients in this product, primary eye irritation testing is required.

Primary Dermal Irritation

In a study, no edema or erythema were observed on the shaved, abraded and intact, skin of rabbits given 24 hours dermal exposure to 0.5 ml of an E.C. containing 65.9% A.I. methoprene (Hepler et al., 1979, 00010915). The primary dermal irritation score was 0 (out of a possible 8 score). At 5 days after treatment, some animals had cracking and flaking skin; by two weeks this condition had cleared. This product is not a primary skin irritant and should be placed in Toxicity Category IV for dermal irritation.

In another study (Hallesy and Hill, 1972, GS0030-001), the primary dermal irritation potential of an E.C. containing 10% A.I. methoprene was evaluated. In this study, female New Zealand albino rabbits were given 24- hour dermal exposure to 0.5 ml of an E.C. containing 10% A.I. methoprene. The test material was applied to the shaved, abraded and intact skin of rabbits under an occlusive wrap. The primary dermal irritation score was 0 out of a possible 8 score. This product is not a primary skin irritant and should be placed in Toxicity Category IV dermal irritation.

No further primary dermal irritation testing is required with the existing E.C. products containing methoprene as their single active ingredient.

Dermal Sensitization

No data were available to assess the dermal sensitization potential of the E.C. formulations containing 5-65.9% A.I. methoprene. Based on an evaluation of the inert ingredients in these products and the available acute data, testing is not required.

Toxicology Profile: Granular and Pelleted/Tableted

No studies were available on the acute oral, acute dermal or acute inhalation toxicities of the granular or pelleted/tableted formulations containing 0.02-7.9% methoprene. Since the Confidential Statements of Formula (CSF) do not indicate an anticipated change in the acute toxicities of these products due to inert ingredients, testing of the technical chemical is sufficient for these products. Therefore, additional testing in these disciplines is not needed.

No studies were available on the primary eye and dermal irritation and dermal sensitization potentials of the granular and pelleted/tableted formulations of methoprene. Based on the CSF's and the available acute data for methoprene, dermal irritation and dermal sensitization are not anticipated with these products, and these tests are not needed. However, eye irritation could result from accidental eye exposure to these products. One eye irritation test is required with a granular product. This test will satisfy eye irritation testing requirements for the existing granular and pelleted/tableted products.

Topical Discussions: Granular and Pelleted/Tableted Methoprene Products

Acute Oral Toxicity

No data were available to evaluate the acute oral toxicity of the granular or pelleted/tableted formulations containing 0.02 to 7.9% methoprene. Since the Confidential Statements of Formula (CSF) do not indicate an anticipated change in the acute oral toxicity of these products due to inert ingredients, testing of the technical chemical is sufficient for this product. Thus, these products should be placed in Toxicity Category IV for acute oral toxicity and additional testing in this discipline is not needed.

Acute Dermal Toxicity

No data were available to evaluate the acute dermal toxicity of the granular of pelleted/tableted formulations containing 0.02 to 7.9% methoprene. Since the CSF's do not indicate an anticipated change i the acute dermal toxicity of these products, due to their inert ingredients, testing of the technical chemical is sufficient. Thus, these products should be placed in toxicity Category III for acute dermal toxicity and additional testing in this discipline is not needed.

Acute Inhalation Toxicity

No data were available to evaluate the acute inhalation toxicity of the granular or pelleted/tableted formulations containing 0.02 to 7.9% methoprene. Since the CSF's do not indicate an anticipated change in the acute inhalation toxicity of these products due to inert ingredients, testing of the technical chemical is sufficient. Thus, these products should be placed in Toxicity Category IV for acute inhalation toxicity and additional testing in this discipline is not needed.

Primary Eye Irritation

No data were available to evaluate the primary eye irritation potential of the granular or pelleted/tableted formulations containing 0.02-79% methoprene. One eye irritation test is required with a granular formulation of methoprene. This test will satisfy the eye irritation testing requirements for the existing granular or pelleted/tableted products.

Primary Dermal Irritation

No data were available to evaluate the primary dermal irritation potential of the granular or pelleted/tableted formulations containing 0.02 to 7.9%

methoprene. Since the CSF's do not indicate an anticipated change in the primary dermal irritation potential of these products due to inert ingredients, testing of the technical chemical is sufficient. Thus, these products should be placed in Toxicity Category IV for primary dermal irritation and additional testing in this discipline is not needed.

Dermal Sensitization

No data were available to evaluate the dermal sensitization potential of the granular of pelleted/tableted formulations containing 0.22 to 7.9% methoprene. Based on an evaluation of the inert ingredients in these products and the available acute data on methoprene, testing is not needed.

Toxicology Profile: Pressurized Liquid (PRL)

No data were available to assess the acute oral, acute dermal and acute inhalation toxicity of the PRL formulation containing 0.15% methoprene. Based on an evaluation of the inert ingredients, testing of the manufacturing use product containing 15% methoprene should be sufficient to fulfill these data requirements. Therefore, separate acute toxicity testing with the PRL product is not needed.

Topical Discussions: Pressurized Liquid (PRL)

Acute Oral Toxicity

No data were available to assess the acute oral toxicity of the PRL formulation containing 0.15% methoprene. Based on an evaluation of the inert ingredients, testing of the manufacturing-use product containing 15% methoprene should be sufficient to fulfill this data requirement. Therefore, acute oral toxicity testing with this product is not required.

Acute Dermal Toxicity

No data were available to assess the acute dermal toxicity of the P.R.L. formulation containing 0.15% methoprene. Based on an evaluation of the inert ingredients, testing of the manufacturing-use product containing 15% methoprene should be sufficient to fulfill this data requirement. Therefore, acute dermal toxicity testing with this product is not required.

Acute Inhalation Toxicity

No data were available to assess the acute inhalation toxicity of the P.R.L. formulation containing 0.15% methoprene. Based on an evaluation of the inert ingredients, testing of the manufacturing-use product containing 15% methoprene should be sufficient. Therefore, acute inhalation toxicity testing with this product is not required.

Primary Eye Irritation

No data were available to assess the primary eye irritation potential of the PRL formulation containing 0.15% methoprene. Since this product contains a propellant, primary eye irritation testing is required.

Primary Dermal Irritation

No data were available to assess the primary dermal irritation potential of the PRL containing 0.15% methoprene. Based on an evaluation of the inert ingredients, testing of the manufacturing-use product containing 15% methoprene should be sufficient to fulfill this data requirement. Therefore, primary dermal irritation testing is not required with this product.

Dermal Sensitization

No data were available to assess the dermal sensitization potential of the PRL containing 0.15% methoprene. Based on an evaluation of the inert ingredients, testing of the manufacturing-use product containing 15% methoprene should be sufficient to fulfill this requirement. Therefore, dermal sensitization testing with this product is not required.

Chapter VII

Residue Chemistry

Residue Chemistry Profil: Manufacturing-use Methoprene

Methoprene is marketed in the U.S. as an insect growth regulator by Zoecon Corporation. Methoprene is sold domestically as an agent for mosquito control. Methoprene is used for hornfly control in combination with cattle mineral supplements. It is also used for the control of cigarette beetles on stored tobacco. A minor use of methoprene is for control of mealy bugs and spider mites on houseplants.

The metabolism of methoprene in plants an animals is adequately defined. The residue of concern is the parent compound. Metabolism studies with plants using ^{14}C-methoprene have revealed the presence of as many as six primary non-polar metabolites in the plant (ZR-669, ZR-724, ZR-725, ZR-1945, ZR-1602 and ZR-1564). Enzymatic hydrolysis studies indicate incorporation of organo-extractable degradation products into glucose and cellobiose. The bulk of any remaining activity is believed to be associated with inert lignins and tannins.

In animals, no additional non-polar metabolites were found. In a study with a steer, the major part (55%-90%) of the non-polar fraction was characterized as ^{14}C-cholesterol. Methoprene is also catabolized to 2-^{14}C-acetate which itself is the precursor of cholesterol in the animal.

Gas liquid chromatography methods using flame ionization detection have been developed for determining residues of methoprene in the various commodities of concern. For enforcement and data collection purposes, these methods are adequate for the commodities covered in this Registration Standard.

Feeding studies, reflecting residues of methoprene in large animals, are adequate in exhibiting the possible transfer of methoprene to fat, meat and milk. The established tolerances for residues of methoprene which may result from the registered uses are adequate for the raw agricultural commodities (40 CFR 180.359). The use of methoprene as a feed additive under specific conditions is regarded as safe (21 CFR 561.282). The use of methoprene in the control of floodwater mosquitoes is exempt from the requirements of a tolerance in potable water when used on pastures, rice fields, marshlands and other non-crop areas (21 CFR 192.285). The use of methoprene to control floodwater mosquitoes is exempt from the requirements of a tolerance in or on the raw agricultural commodities fish, forage grasses, forage legumes, rice, rice straw and shellfish when used on pastures and rice fields, and marshlands and other non-crop areas (Federal Register Notice of December 4, 1981, Volume 46, No. 233, p. 59248).

Topical Discussions

Uptake, Distribution, and Metabolism in Plants

The metabolism and paths of degradation of Altosid in plants have adequately been defined.

The elucidation of the metabolism of methoprene in plants started with a 3H-labelled methoprene study with the dwarf lima bean plant. This study indicated the presence of metabolites ZR-724 and ZR-725 in addition to the parent component in foliar parts of the plant (see Table 1 of this Chapter).

An additional study was performed with ^{14}C-methoprene in the alfalfa plant. Leaves of 3-month old plants were painted with the labelled material and the treated leaves sampled at various intervals up to 30 days. The recovery of unmetabolized methoprene followed a first-order decay plot with a half-life of about 2 days. After 30 days, 1% of the applied activity remained as methoprene. The amount of recoverable ^{14}C-label decreased exponentially as a function of time. The appreciable loss of activity initially (20% in one day) could be attributed to evaporation from the leaf surface.

Three principal non-polar metabolites were identified from a chloroform extract of the plants: ZR-725, ZR-1945 and ZR-724. These metabolites were produced in essentially equal amounts (combined 8.8% of the applied dose) with maximum accumulation during the third day. The major means of plant metabolism are believed to involve ester hydrolysis to give ZR-725 with some oxidative dimethylation to form ZR-724. ZR-1945 is thought to arise from photoxidation of the double bond of the dienoate on the leaf surface.

Enzymatic hydrolysis (cellulase and sulfatase) of the polar conjugate in a methanol extract produced mainly ZR-724 (7.4% of applied) plus a smaller amount of ZR-725 (2.2%). Lesser amounts (1%) of the conjugated photo-product (ZR- 1954) were also isolated.

Combustion of the plant tissue remaining, after exhaustive extraction with methanol and chloroform, revealed some residual activity. A maximum of 9.7% of the total dose was found at 30 days in the unextractable portion. This bound activity is believed to be attributed to decomposition products which are incorporated into cellular constituents. Extraction of the untreated portions of the plant showed a maximum accumulation of 0.6% of the total activity after 15 days. This activity is thought to be the result of transport of degraded polar metabolites rather than the translocation of Altosid *per se*. No detectable activity was found in the roots. Translocation studies showed no evidence of any uptake of residues into wheat plants grown in soil treated with ^{14}C-Methoprene.

A similar study with rice plants using 5-^{14}C-methoprene has revealed the presence of six primary non-polar metabolites in the plant (ZR-669, AR-724, ZR-725, AR-1945, ZR-1602 and ZR-1564). The major aglycone from rice was AR-1945 (1.2%) with a small amount of ZR-1602 (0.3%). A half-life of about 1/2 day was observed on rice. After 39 days, only 0.4% of the applied dose remained on rice as unmetabolized methoprene. After 15 days on rice, the majority of the residual activity was either highly polar (57% at origin of TLC) or unextractable with methanol 38%.

Considerable activity apparently incorporates into natural plant constituents. Gel permeation and TLC evidence suggest that chlorophylls, carotenoids, and unknown high (1000) molecular weight substances had incorporated ^{14}C-label. Enzymatic hydrolysis of the cellulosic pulp remaining after organic extraction indicated incorporation of activity into glucose and cellobiose. The bulk of any remaining activity is thought to be associated with inert lignins and tannins.

These studies indicate that these metabolites are formed by ester hydrolysis, O-demethylation and oxidative scission of the 4-ene bond. The distribution of the metabolites depends on the type of the plant. Methoprene is eventually degraded to acetate and may be used in the synthesis of plant constituents.

The metabolism of methoprene in plants is considered to be adequately defined and no additional data are needed.

Metabolism in Animals

In metabolism studies with the mouse using ^{3}H-methoprene, most of the administered activity (82%) was eliminated in the urine and feces after 96 hours. Small amounts of activity were found in the tissues. Three groups of metabolites were chromatographically separated in the urine: a glucuronide type conjugate; a mercapturic acid conjugate, and finally a complex mixture of apparent bound free acids.

Some further metabolism studies involving the feeding of ^{14}C-labelled methoprene to the guinea pig were reviewed. After 24 hours, 40% of the administered activity was found in the urine, 8% in the feces and 17% eliminated as expired CO_2. Although the study was conducted for only 24 hours, the ratio of urinary to fecal metabolites was about the same as that found in the mouse study. The study was terminated after 24 hours because it was designed as a pilot study for a steer metabolism study.

Accordingly, a steer was given a single large dose (equivalent to about 240 ppm in the total diet) of 5-^{14}C-methoprene and selected tissues (fat, muscle, liver lung and blood) were analyzed for methoprene and its metabolites. Only trace amounts (0.01 ppm) of methoprene or its known primary metabolites were detected. The major (55-90%, depending upon tissue) portion of the non- polar fraction was characterized as ^{14}-cholesterol by chemical derivatization. A lipophilic fraction (less polar than methoprene) was also found (11-30%). Due to the high dilution of radiolabel by extracted lipids, it was not possible to investigate the identity of this non-polar material in the solid tissues. However, from blood extracts it was possible to identify the radiolabelled lipid as ^{14}-C cholesterol ester of linoleic, palmitic and other unientified fatty acids, with 98% of the radiolabel associated with the sterol and 2% associated with the fatty acids. Liver tissue that had been exhaustively extracted with organic solvents was subjected to enzymatic hydrolysis with Pronase in an attempt to solubilize and identify unextractable radioactivity. This treatement did solubilize 83% of the bound label; however, 90% of this solubilized activty was water soluble and believed to be associated with amino acids. Further investigation of the individual amino acids was not feasible due to the low concentration of activity in the extracted tissue.

Additional tracer studies with a lactating cow show that methoprene was extensively metabolized to acetate which was isolated from blood as randomly-labelled acetic acid. Radioactive acetate was incorporated into milk fat which was degraded to radiolabelled saturated monoenoic and dionoic fatty acids. Radioactive lactose, lactalbumin and casein were also isolated from milk. The presence of ^{14}C-cholesterol (free and esterified)was confirmed in blood, in agreement with the previous study in a steer. Additional tracer studies with the chicken were performed to determine the fate of methoprene on poultry. The major pathways of excretion of labeled material are feces, urine, and expired CO_2, which account for 90% of the administered dose. No new metabolites of methoprene were detected in the chicken. As with the cow, a large part of the activity in fat resulted from degradation of parent compound to acetate which was then incorporated into natural products (fatty acids). In chicken muscle (breast) most of the activity was associated with residual solids (protein). In the egg, the majority of the activity was in the yolk and consisted of nonpolar material believed to represent radiolabeled lipid.

The metabolism of methoprene in animals is considered to be adequately defined and no additional data are needed.

Metabolism in Water

Studies available on the degradation of methoprene in water using both ^{3}H and ^{14}C material permit these conclusions (Schooley et al., 1975, 05008622):

1) Methoprene is stable to chemical hydrolysis for over 4 weeks in the dark.

2) The dienoate moiety is readily attacked and degraded by photocatalyzed oxygenation. ZR-1945 is the major breakdown product.

3) Methoprene decomposes in water containing active microorganisms to ZR-724, ZR-725 and ZR-669 by combined ester hydrolysis and oxidative demethylation.

4) The transisomer is converted to an essentially equal (1:1) mixture of cis and trans-isomers in a few hours in water exposed to sunlight. The indicated half-life for methoprene in pond water is about 1.5 to 2.0 days.

Analytical methodology

Since methoprene per se is the only chemical of concern to be analyzed, the methods for identification and quantification are practically the same. Small modifications have been made to the basic method to accomodate for differences in the various commodities to be analyzed. These modifications most frequently occur in the extraction and clean-up phases of the method.

The basic analytical procedure consists of extracting the methoprene from the sample material. The extract is diluted with water and methoprene is partitioned into petroleum ether. Next residues of methoprene are isolated from coextracted substances by chromatography on a Florisil column followed by chromatography on a neutral alumina column, eluting with mixed petroleum and diethyl ethers in each case. Residues of methoprene in concentrated eluates are measured by gas chromatography utilizing a hydrogen flame ionization detector. The column consists of a 2M x 2mm (i.d.) pyrex column packed with detector. The column consists of a 2M x 2mm (i.d.) pyrex column packed with 3% w/w methyl silicone (OV-101) on 100/120 mesh Chromasorb W-AW-DMCS and conditioned at 280°C until a stable base line is attained.

Method #009A, "Residue determination of Altosid insect growth regulator in forage grasses, forage legumes, rice foliage and grains (rice, wheat and other)" was used in the analysis of methoprene residues in grain, legume forages, rice foliage and grain (rice and wheat). Recoveries obtained for Methoprene on the above commodities range from 80-110%. The limit of detection was 0.05 ppm for the crops. method #009A was also used in the anlaysis for detecting methoprene residues in water. Water samples were diluted with water and residues partitioned into petroleum ether. Concentrations of 0.01 ppm or higher are determined directly without further clean-up. Residues are calculated and reported in terms of the trans isomer only. Recoveries of residues in water ranged from 85-115%. The limit of detection was reported to be 0.001 ppm.

Several confirmatory methods for methoprene are available including a TLC procedure, a combined CC-MS techniques and one involving the extraction of p-values.

It is concluded that the methods for water and crops are adequate for enforcement purposes and the collection of residue data for methoprene in crops (i.e., forages and rice).

In addition, there is similar method (Method #067) "Residue Determination of Altosid Insect Growth Regulator in Cows Milk" suitable for the analysis of residues of methoprene in milk. This method was tried out in one of the EPA laboratories and was found satisfactory for the purposes intended. A 50 gram sample of milk was fortified at 0.01 ppm and 0.02 ppm. Recovery ranged from 0-110 ppm. The sensitivity of the method is regarded as 0.005 ppm.

A similar method (Method #068) "Residue Determination of Altosid Insect Growth Regulator in Animal Tissues and Blood" is suitable for the analysis of residues of methoprene in animal tissues (including poultry) and/or fish and shellfish. Also, this method was tried our and found adequate for the purposes intended. A sample of 25 grams of beef fat was fortified with 0.10 ppm and 0.24 ppm of methoprene. Recoveries ranged from 88-110%. The sensitivity of the method is regarded as 0.02 ppm.

Method #032 is available for the analysis of methoprene residue in eggs. Recoveries are reported as 85-120%. The limit of detection is 0.01 ppm. For residues of methoprene per se, it is concluded that these methods are adequate for enforcement purposes and for the collection of residue data for commodities pertaining to this Standard.

For identification purposes, a GC method (#401) was also developed to check animal tissues for residues of several of the known metabolites (ZR-669, ZR-724, ZR-725). Residues are isolated by double extraction with an acetonitrile-sodium sulfate-celite mixture. Cold temperature precipitation and filtration are used for removal of fat. Specific metabolites are isolated by partitioning with petroleum and diethyl ether. The carboxylic acid metabolites (ZR-724 and 725) are methylated, and the derivatives partitioned into petroleum ether. After Florisil-alumina column cleanup, GC (flame ionization) is used for quantitation. The limit of detection is 0.01 ppm for each component. No recovery data were available.

Pesticide Analytical Manual, Volum II, Section 180.359, reports several analytical methods for methoprene for regulatory and enforcement purposes.

No additional information on the analytical methods is needed.

Residue Data

Field residue data for methoprene should reflect the registered uses in regard to application rate, modes of application, number and timing of treatment, formulations used and geographical area represented. The analytical methods used to generate the residue data on the raw agricultural commodities subject to this Standard are practically the same as those used for regulatory purposes. These analytical methods are regarded as adequate for data generation.

Storage stability tests indicate no appreciable decomposition of methoprene in samples of water, soil and crops (clover and pasture grass) when held at -20°C for periods up to 63 days (Zoecon, 1973, 00010384).

The technical material consists of about 93% trans isomer with about 3% cis isomer. Of the remaining 4%, about 3% consists of related impurities which are identified and about 1% unidentified. No residue problem is anticipated from these impurities.

The residue data are presented in this Chapter by the use pattern from which the residues are likely to result: use of methoprene for mosquito control in floodwater, use of feed supplements to cattle and dairy cows, and on mushrooms.

Residues of methoprene resulting from the use of mosquito control in floodwater

Methoprene is applied at the rate of 0.02-0.03 lb a.i./A in pastures, rice fields and certain non-crop areas. For adequate penetration of dense vegetation in non-crop areas, up to 0.04 of a.i./A may be used. The formulation is applied to second, third, or fourth stage larvel instars of flood water mosquitoes in 0.5 to 50 gallons of water per acre using suitable air or ground equipment to insure good coverage. There is a restriction against treatment of rice after heading (Steelman, 1972, 00011485; Zoecon, 1973, 00011493; Zoecon, 1973?, 00010826; Zoecon, 1973, 00010823).

Forages

All studies at the recommended treatment rate show residues of methoprene in forages (grasses and legumes) of 0.2 ppm or less both initially and at later intervals (1 to 9 days after treatment) (Washino, 1973, 00010827; Meisch, 1973, 00010374; Meisch, 1973, 00012782).

Initial residues up to 2 ppm were reported from exaggerated treatments (2-4X). There is a rapid decline in residues shortly after treatment to levels below 0.1 ppm after 2 to 3 days. It is concluded that a tolerance of 0.5 ppm for forages is adequate.

Rice

Methoprene is applied on rice when the rice is irrigated early in the season by standard flood irrigation methods. There is a built-in preharvest interval (PHI) of least two months because of this usage pattern.

This is considered essentially a "no-residue" situation and this is supported by the data which show no detectable residues (0.005 ppm) in rice grain (including hulls) and rice straw in any of the samples analyzed (Steelman, 1972, 00011485; Zoecon, 1973, 00011493).

Since no detectable residues are present in rice grain, there will be no problem with residues in milled fractions.

Water

In studies with air and ground application of methoprene to standing water in pastures, irrigated rice fields and marshlands at 1 to 40X the recommended rate, no detectable residues (0.001 ppm) were found in water 2 days or longer after treatment (Zoecon, 1973, 00010371).

In this study, one value of 0.25 ppm (probably spurious) was reported at the 2X rate immediately after treatment, but this had declined to 0.003 ppm at 1 day and to non-detectable amounts (0.001) at 3 days. The calculated initial concentration of methoprene is 0.015 to 0.03 ppm (based on water depths of 6 in. to 1 ft. which is normal in cases where methoprene is applied to standing water).

Also, in this study the movement of residues in water was considered. A lagoon in a drainage system was sampled at points up to 1,000 ft. downstream for a period of 7 days after treatment. Treatment rate was 40X the recommended rate. At the point of application, initital residues of 0.18 ppm decreased to 0.005 ppm after 1 day, to 0.002 ppm at 3 days, and to non-detectable amounts thereafter. At the various points sampled 100, 300 and 1,000 ft. downstream from the treatment site, residues were in all cases 0.006 ppm or less at the collection intervals of 1 hour to 7 days after treatment.

From these results, it is concluded that a maximum residue level of 0.01 ppm in potable water is appropriate.

Other Crops

The use of methoprene to control floodwater mosquitoes is limited to situations where water stands for a sufficient time and provides an environment for mosquito breeding. Treatments are limited to pastures, rice fields, and certain non-crop areas such as marshlands. Therefore, there will be no direct treatments to any crops other than forages and rice.

Situations in which methoprene-treated water enters into an irrigation system are unlikely. However, if such a situation occurs no detectable residues would be expected in irrigated crops due to the dissipation rate of methoprene in water (Zoecon, 1973, 000010371).

Meat and Milk

Lactating cows fed methoprene at levels up to 1.0 ppm in the total diet for 28 days showed non-detectable residues (0.01 ppm) in milk except for one value of 0.012 ppm found at the highest (1.0 ppm) feed level. Residues in meat (excluding fat) tissues were non-detectable (0.01 ppm) except for trace residues (0.02 ppm) in liver and kidney. Residues in fat up to 0.09 ppm were found at the highest (1.0 ppm) feed level.

Additional data were obtained through feeding steers at very high rates (30 to 300X) in connection with another contemplated use of methoprene in the control of fecal breeding flies. Residues in non-fat tissues at these exaggerated feed rates were in all cases 1.0 ppm. Residues in fat up to about 7.9 ppm were found at the maximum (300X) rate. Both the low-level feeding studies and those at the highly exaggerated rates support the adequacy of a 0.1 ppm tolerance for meat tissues, including fat.

It is concluded that a tolerance level of 0.01 ppm for milk and 0.1 ppm for meat resulting from the registered use of floodwater treatment are appropriate. These conclusions for cattle can be applied to other livestock animals (hogs, horses, sheep and goats).

Poultry and Eggs

Residues in poultry tissues and eggs could be as a result of use of methoprene on rice and possible residues in the poultry drinking water. In either case, only low-level (0.01 ppm) concentrations of methoprene would be present.

In one study, birds were fed 25-50 ppm in the diet. Slight residues of 0.1 ppm or less were found in the tissues and 0.05 ppm or less in eggs (Zoecon, 1973, 00010904; Zoecon, 1974, 00011106; Morgan, 1973, 00011130; Zoecon, 1974, 00011109; Burd, 1973, 00010837; Jensen, 1973, 00010838).

It is concluded that a tolerance of 0.1 ppm for poultry tissues and eggs is appropriate.

Fish and Shellfish

Residue data are available for methoprene for fish and shellfish. Studies were conducted with fish using caging systems and/or man-made ponds stocked with the various fish species (Zoecon, 1973, 0012787). Top, middle, and bottom feeders (sunfish, catfish, bass, trout, bluegills, and shiners) were included in the study.

Sampling intervals up to 7 days after treatment were reported. (Tracer studies with ^{14}C methoprene at a low level approximating the proposed use and at a high (25X) level showed maximum total residues by activity measurement at about 3 days after treatment with a decline in residues by 7 days). In one study in a California lake, bluegills were exposed to two treatments with unlabeled material at 10X the maximum recommended rate. Residues peaked at 1.3 ppm at 1 day after treatment and declined to <0.2 ppm after 7 days. Residues in shellfish (clayfish and crab) from salt marshes treated at 1 to 2X recommended rate with unlabelled methoprene showed only trace residues (0.02 ppm) in the edible portions.

From the results, it is concluded that residues of Mthoprene would not exceed a tolerance level of 0.1 ppm in fish and shellfish.

The above discussion of residues in various commodities which may result from the registered uses indicated the tolerance levels which are appropriate to cover those registered uses. However, Zoecon Corporation requested an exemption from the requirement for residue tolerances on the commodities in question.

Decisions on the granting of an exemption are normally based on toxicological considerations. Accordingly, the Agency granted an exemption from the requirements of a tolerance for methoprene in forage grasses and legumes, rice and rice straw, meat, eggs, milk, fish and shellfish and potable water. This exemption is limited specifically to the use of methoprene on pastures, rice fields, marshlands, etc., to control floodwater mosquitoes. (See Title 21 part 193 Food Additives Section 193.285. pertaining to potable water and Title 40 part 180 Section 180.1033 pertaining to raw agricultural commodities.)

Residues of methoprene in raw agricultural commodities as a result of the use of feed supplements to cattle and dairy cows.

Methoprene is added to mineral blocks or other feed supplements which are fed to cattle and dairy cows on a free choice basis for fecal control of horn flies. To be effective, the formulation must be incorporated into the supplement to allow daily intake of about 7.5 mg. of active ingredint per 100 lb of supplement. The recommended treatment rate is equivalent to a daily intake of 0.0165 mg. a.i./kg. body weight or 22.7 to 45.4 mg. per 100 lb of body weight per month. No withdrawal period between treatment and slaughter is indicated.

Meat and Fat

In cattle feeding studies previously discussed in this chapter cattle were fed methoprene at levels up to 1.0 ppm in the total diet. This is equivalent to about 2X the recommended field use rate. Residues of methoprene in meat (excluding fat) tissues were non-detectable (<0.01 ppm) except for trace residues (0.02 ppm) in the liver and kidney. Residues in the fat of up to 0.09 ppm were reported.

In other studies using feed rates of 0.016, 0.048, and 0.16 mg/kg of methoprene (equivalent to 1, 3, and 10X the recommended rate), maximum residues in omental fat (the site of maximum residues as shown by the earlier studies), were about 0.3 ppm from the highest feed level (Moorman, 1974, 00010593). Prolonged feeding up to 21 weeks indicated that a plateau is reached after about 4 weeks. Studies on the decline in residues upon withdrawal from feeding of methoprene reveal a fairly rapid decrease in residues with an indicated half-life of about 1 to 2 weeks.

In addition to the controlled feeding studies, field tests were conducted in which cattle were exposed to mineral blocks containing methoprene at concentrations approximating the recommended use. The animals were omenectomized, without a withdrawel period, after 4 or 5 weeks of continued exposure to the treated block. Samples of omental fat were excised and analyzed for methoprene content. Results from two such studies using 5 animals in each case show that mean tissue residue values can be predicted from the average consumption of methoprene and, also, that in the most variable case, individual animal consumption will not differ by more than a factor of 2 from the mean herd consumption. The maximum residue found in these free choice studies was 0.13 ppm, with average residues of 0.08 to 0.09 reported.

In summary, controlled feeding studies indicate that residues of methoprene in all tissues (including fat) are below 0.1 ppm from feeding rates equivalent to 2X the recommended field use rate. Free choice experiments also indicate that residues in the tissues would be less than 0.1 ppm under the proposed conditions of use. Even in the case where an individual animal was to feed at twice the designed consumption rate, residues would not normally exceed 0.1 ppm. In all cases, residues in all tissues would be below 0.1 ppm after 14 days exposure to methoprene in the feed.

It is concluded that residues in tissues (excluding fat) would be non-detectalbe (0.01 ppm) except for trace residues (0.02 ppm) in liver and kidney without a withdrawal period. Residue in omental fat at zero days could result up to about 0.15 ppm. Accordingly, a tolerance of 0.3 ppm in fat, which was requested by Zoecon Corporation, is appropriate. (Note: it is accepted practice to establish a tolerance in terms of fat only in cases such as these where a higher tolerance is needed for fat than for meat and meat by-products).

Milk

Methoprene, used in a dietary supplement to control horn flies, is designed specifically for beef cattle on the range. Most dairy cows are kept under essentially a feed lot type of situation where their diet is carefully controlled for maximum productivity. In this situation the horn fly is not a problem. Nevertheless, some dairy farmers might in certain cases administer methoprene in a block mineral feed to grazing dairy animals.

On a controlled study, lactating dairy cows were fed methoprene at 1.0 ppm in the total diet (about 2X the field use rate) for 28 days (Zoecon, 1978, 00010895). All residues in milk were reported as non detectable (0.01 ppm) except for one value of 0.012 ppm which was probably spurious since no methoprene was detected in this animal's milk in any of the other samplings. In studies conducted at 75X the recommended feeding rate, positive residues were detected in milk; the maximum residue was 0.7 ppm in one case but most values were 0.5 ppm or less. As is the case in tissues, there is a rapid decline in residues in milk upon withdrawal with an indicated half-life of about 2 to 3 days.

However, other controlled studies conducted at 1.5, 5 and 15 times the recommeded rate indicate that maximum extropolated residues in excess of 0.01 ppm (up to 0.03 ppm) might occur in some cases (Zoecon, 1976, 00010889).

It was reported that intake studies on a free choice basis were performed on lactating cows in pasture as well as on steers and heifers. These studies conformed that individual animal consumption would not differ by more than a factor of 2 from the mean herd consumption. The concern is whether the tolerance level for milk is adequate in covering cases where an individual animal might consume excessive amounts of the treated mineral block while on pasture. It is also noted that this problem is alleviated to some extent by the fact that milk is pooled during collection, pasteurization and homogenization so that residue levels in milk from an individual animal are not critical.

On the basis of all of these considerations, it is concluded that a tolerance of 0.05 ppm for milk is appropriate.

Mushrooms

Methoprene is to be applied in single or double applications to control sciarid flies in mushroom cultures.

The methoprene formulation used on mushroom cultures is mixed with water and sprayed within 48 hours of mixing. The volume is dependent on the conditions of casing and the compost. The use of methoprene for mushrooms is as follows:

Single Application. Incorporate into casing at time of casing. Apply methoprene at the rate of 3.4 oz. a.i. per 1000 square ft. Make application evenly and mechanically mix into casing material prior to the casing operation or drench evenly onto the surface of the casing immediately after placement.

Double Application. Incorporate into compost at time or spawning and in casing at time of placement of casing layer.

In compost:

Apply methoprene at the rate of 3.4 oz ai per 1000 sq. ft in adequate water for even distribution. Spray or sprinkle on the surface of compost at the time of spawning and thoroughly incorporate using a spawning machine or other suitable mechanical means.

In casing:

Apply methoprene at the rate of 2 1/4 fluid oz (1.4 oz ai) per 1000 sq. ft. Make application evenly and mechnically mix casing material prior to the casing operation, or drench evenly onto the surface of the casing immediately after setting it in place.

The residue data submitted were obtained by the method recommended for enforcement purposes. Controls were 0.05 ppm for the mushrooms except for one value of 0.07 ppm. The limit of detection was 0.05 ppm. The recovery was reported as 98% (Miller, 1979, 00011036).

Residue data reflect both types of proposed uses at application rates from 0.2 to 4.6X the recommended rates of 10 to 40 gallon/1000 square ft. The data reflect seven residue studies, four from California, two from Pennsylvania and one from Florida (Zoecon, 1979, 00012796). PHI's varied from 9 to 35 days. In the seven studies, the compost and casing layers were treated at various rates. Residue data show that up to 2.5 times the combined treatment rates (casing and compost) residues in the mushrooms are undetectable (<0.05). Between 2.5 and 4.6 times the registered treatment rate, residues start appearing in the mushrooms (i.e., at 4.6 times the use rat, methoprene residue in mushrooms were 0.17 ppm); casing residues in this case were very high (150 ppm). Overall residues in the compost and casing were reported from 0.05 ppm to 150 ppm.

Other studies at higher application rates (1.6 oz ai/100 sq ft), applied as a drench to the compost surface 1 to 24 hours prior to adding the casing, show significant residues. No residue data reflecting unwashed mushrooms were submitted from this use (Zoecon, 1980, 00001240). In a similar study where methoprene was applied to the casing layer at 1.6, 3.2, and 6.2 oz a.i./100 sq. ft., residues, at 23 to 49 PHI's, ranged from 1.46 to 2.66 ppm, 2.68 to 4.51 ppm and 5.71 to 7.4 ppm respectively.

Considering the registered uses, it is concluded that the residue data indicate that the established 1 ppm for residues of methoprene in mushrooms is appropriate and adequately supported.

Tolerances

Tolerances for residues of methoprene have been established for the following raw agricultural commodities (40 CFR 180.359 as amended in FR Vol. 46 No. 233, Friday, December 4, 1981).

Milk	0.05 ppm
Mushrooms	1.0 ppm
Cattle, fat	0.3 ppm
Cattle, meat	0.1 ppm
Cattle, meat by-products	0.1 ppm
Eggs	0.05 ppm
Goats, fat	0.3 ppm
Goats, meat	0.1 ppm
Goats, meat by-products	0.1 ppm
Hogs, fat	0.3 ppm
Hogs, meat	0.1 ppm
Hogs, meat by-products	0.1 ppm
Horses, fat	0.3 ppm
Horses, meat	0.1 ppm
Horses, meat by-products	0.1 ppm
Peanuts	2.0 ppm
Peanut hulls	40.0 ppm
Poultry, fat	0.5 ppm
Poultry, meat	0.5 ppm
Poultry, meat by-products	0.05 ppm
Sheep, fat	0.3 ppm
Sheep, meat	0.1 ppm
Sheep, meat by-products	0.1 ppm

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This amendment replaces the exemption for the requirement of a tolerance for methoprene in or on raw agricultural commodities with tolerance levels set forth in 40 CFR 180.1033. The commodities fish, forage grasses, forage legumes, rice, rice straw and shellfish remain exempted from the requirement of a tolerance under 40 CFR 180.1033.

Methoprene as a feed additive may be safely used in accordance with the following prescribed conditions (21 CFR 561.282):

(a) It is used as a feed additive in the form of mineral and/or protein blocks or other feed supplements in the feed of cattle at the rate of 22.7 to 45.5 milligrams per 1000 pounds of body weight per month.

(b) It is used to prevent the breeding of horn flies in the manure of treated cattle.

(c) To ensure safe use of the additive, the label and labeling of the pesticide formulation containing this additive shall conform to the label and labeling registered by the U.S. Environmental Protection Agency.

Canada has established no tolerances for methoprene. Methoprene is cleared for use on pastures in Canada. Mexico has no tolerances for methoprene and there are no Codex tolerances.

Chapter VIII

Ecological Effects

Ecological Effects Profile: Manufacturing-Use Methoprene

Data on the toxicity of methoprene to avian species, freshwater fish and invertebrates, amphibians, and estuarine and marine invertebrates are available.

Methoprene was found to be practically non-toxic to mallard ducks with an acute oral LD_{50} value >2000 mg/kg and an 8-day dietary LC_{50} of >10,000 ppm. The LC_{50} value for methoprene in bobwhite quail was found to be P10,000 ppm. Methoprene had no effects on quail reproduction at dietary concentrations of 30 ppm. Dietary concentrations of 30 ppm caused some reproductive impairment in mallard ducks, but concentrations of 3 ppm had no effect.

Methoprene can be characterized as very highly toxic to freshwater invertebrates. The 48-hour EC_{50} of technical methroprene to Daphnia magna was 89 ppb and the 42-day maximum acceptable tolerance limit was calculated to between 27 and 51 ppb. Although technical methoprene was found to be highly toxic to Daphnia magna, it was found to be only moderately toxic to a wide variety of other aquatic invertebrates. Crayfish exposed to aged radiolabeled methoprene residues showed some tendency to accumulate the radiolabel, but only to about 66 times the ambient water concentration and to levels equal to the ambient soil concentration.

Methoprene is moderately toxic to warm water, freshwater fish. The 96-hour LC_{50} to blue-gill sunfish was found to be 1.52 ppm. It was found that concentrations above 0.624 ppm reduced the blood serum glucose levels of exposed rainbow trout, but no valid data are available on the toxicity of methoprene to cold water fish.

Under laboratory conditions, the edible portions of bluegill sunfish accumulated 550 and 950 times the ambient water concentrations of 0.005 and 0.31 ppm before reaching a plateau. Non-edible portions of the fish contained residue levels 12 times and 4 times greater than edible portions in the low and high concentrations, respectively. Almost 90% of the residue was found to be unmetabolized methoprene and fish removed from exposure excreted 93-95% of the residue with 14 days. Under simulated natural conditions, bluegills accumulated substantial amounts of the radiolabel, but only 4-5% of the total ^{14}C-residue was found to be methoprene or its metabolites.

Technical methoprene is moderately to slightly toxic to amphibians. A variety of toad, frog, and salamander larvae were tested and 96-hour LC_{50} values ranged from 1.0 to >10 ppm.

Technical methoprene is highly toxic to larvae of estuarine and marine invertebrates, but only slightly toxic to adults. Methoprene is also highly toxic to larval mud-crabs, slightly toxic to adult grass shrimp, and highly toxic to juvenile grass shrimp. Technical methoprene may cause inhibition of gametogenesis in mud-crabs exposed to 1.3 ppm for 12-15 days.

A single study, using a material assumed to be technical methoprene indicated that a 600 ppb solution significantly increased chlorophyll-a content, the ratio of heterocysts to vegetative cells, and nitrogen fixation in Aphanizomen, a blue-green alga.

Topical Discussions

Effect on Birds

Avian acute oral LD_{50} and dietary data on either wild waterfowl or upland game birds are required. These avian acute toxicity data are available for methoprene:

Species	Test Substance	Result	Citation
Mallard Duck	Technical Methoprene	LD_{50}>2000 mg/kg	Hundson, 1975, 00010633
Mallard Duck	Technical Methoprene	LC_{50}>10,000 ppm (8-day dietary test)	Fink, 1972, 00012754
Bobwhite Quail	Technical Methoprene	LC_{50}>10,000 ppm	Fink, 1972, 00012755

There is sufficient information to characterize technical methoprene as practically non-toxic to waterfowl and upland game birds. No additional acute toxicity testing is needed.

An avian reproduction study on one species of upland game bird (preferably the bobwhite quail) and one wild waterfowl (preferably the mallard duck) is required because the major use patterns of methoprene called for repeated applications duirng bird breeding seasons.

Two reproduction studies are available; the results are:

Species	Test Substance	Result	Citation
Bobwhite Quail	Technical Methoprene	NOEL > 30 ppm	Fink & Reno, 1973, 00010634
Mallard Duck	Technical methoprene	No effect at 3 ppm some repro. impairment at 30 ppm	Fink & Reno 1973, 00010635

Reproductive impairment noted among mallard ducks fed dietary concentrations of 30 ppm methoprene is significant but not of conern since 30 ppm is approximately 1500 times the estimated maximum exposure rate. Also, methoprene has not shown any tendency to bioaccumulate in birds or their food.

No additional chronic toxicity testing is needed.

Effects on Fish

The minimum data required for establishing the acute toxicity of methoprene to fish are results from two 96-hour studies with technical methoprene, one using

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An adequate 96-hour LC_{50} study is available for warmwater fish. The 96-hour LC_{50} to bluegill sunfish was found to be 1.52 ppm, which characterizes methoprene as moderately toxic to warmwater fish (Sleight, 1972, 00010388).

A study conducted on rainbow trout found that concentrations above 0.625 ppm reduced blood serum glucose levels of exposed rainbow trout (Madder and Lockhardt, 1978, 05008617). However, data were not presented on the 96-hour LC_{50} of technical methoprene to coldwater fish. A 96-hour study on cold water fish is required.

Two ^{14}C-residue bioaccumulation studies are available on bluegill sunfish. Under laboratory conditions of a flow-through study, the mean nominal concentration of radiolabeled residue in edible portions of the fish were 550 and 950 times ambient concentrations of 0.005 and 0.31 ppm, respectively, (Quistad et al., 1976, 05008611). Levels in the non-edible portions of the fish were 12 and 4 times higher than in edible portions for the low and high ambient concentrations, respectively. Almost 90% of the residue was found to be unmetabolized methoprene. Fish removed from methoprene exposure and placed in uncontaminated water excreted 93-95% of the residue within 14 days.

Under simulated natural conditions, bluegills accumulated substantial amounts of the radiolabeled residue, but only 4-5% of the total ^{14}C-residue was found to be methoprene or its metabolites (Staiger et al., 19??, 00010390). Most of the residue in fish exposed for 28 and 42 days consisted of highly polar, extractable compounds. The methoprene appeared to have been degraded at least partially before ingestion by the bluegills. The bluegills then quickly incorporated the radiolabel into natural products.

Effects on Aquatic Invertebrates

The minimum data required for establishing the acute toxicity of methoprene to freshwater invertebrates is a 48-hour EC_{50} study with technical methoprene, using a freshwater invertebrate (preferably Daphnia magna). The 48-hour EC_{50} of technical methoprene to Daphnia magna was determined to be 89 ppb (LeBlanc, 1975, 00010856). In a 42-day chronic study, the maximum acceptbie tolerance limit (MATL) was estimated to be between 27 and 51 ppb (LeBlanc, 1975, 00010856). In a related study, methoprene caused no observable morphological abnormalities and did not hinder the growth of Daphnia magna at sublethal levels (Zoecon, 19??, 00010857). This information is sufficient to characterize methoprene as very highly toxic to aquatic invertebrates.

Under laboratory condtions, methoprene was moderately toxic to a wide variety of aquatic invertebrates, highly toxic to Daphnia magna, and very highly toxic to larvae of the mosquito, Aedes nigromaculis (Miura and Takahaohi, 1973, 05009929). In a field study by Norland and Mulla (1975, 05019974), repeated applications of methoprene to experimental ponds significantly reduced mayfly, chironomid, and dytiscid beetle populations, but did not affect odonates or ostracods. McAlonan (1976, 000010944) found that repeated applications of Altosid SR-10 to artificial salt-marsh ponds had no effect on experimental populations of killifish, grass shrimp, and fiddler crabs.

Crayfish placed in a water and sediment system that had been treated with methoprene and aged 30 days accumulated the radiolabeled residues up to approximately the same concentration as was found in the sediment or about 66 times the concentrations in the ambient water. The accumulation of this concentration took about 14 days and remained to the end of the 42-day study (Sleight, 1973, 00010903).

Effects on Estuarine and Marine Organisms

The minimum data required for establishing the acute toxicity of methoprene to estuarine and marine organisms are acute toxicity tests on an estuarine or marine vertebrate. Testing is required on methoprene because the use pattern indicates direct application to estuarine or marine systems.

No acute toxicity data on marine or estuarine vertebrates are available. Additional data are not required since data are available that indicate methoprene is moderately toxic to freshwater fish and amphibians.

The following data are available on the toxicity of methoprene to estuarine or marine invertebrates:

Species	Test Substance		Result	Citation
1. Eastern Oysters (larvae)	Technical Methoprene	(a)	NOEL >0.075 ppm	Sleight, 1972, 00010851;
		(b)	48-hr TL_{50}= 0.247 ppm	Sleight, 1973, 00010852
2. Larval Mud-crabs	Technical Methoprene	(a)	Lethal at concentrations of 1.0 ppm	Costlow, 1985, 05010827;
		(b)	Concentrations of 0.01 and 0.0001 ppm reduced survival among larvae exposed to low salinity; concentrations of 0.1 ppm reduced survival among larvae exposed to high salinity;	Costlow, 1977, 00010860 Christiansen et al., 1977, 05009394
3. Adult Grass Shrimp	Technical Methoprene	(a)	96-hr LC >10 ppm	Petrocelli, 1975 00010859
		(b)	3.8 ppm resulted total mortality after 7 days	
		(c)	35-day LC_{50} = 972 ppb	

4. Mud-crabs	Technical Methoprene	(a)	Ingestion and topical application of methoprene at 1.3 ppm causes progressive inhibition of vitellogenesis and stimulation of spermatogenesis to mud-crabs exposed 12 to 15 days.	Payen and Costlow, 1977 05010246
5. Larvae Mud-crabs	Technical Methoprene	(a)	Concentrations of up to 0.1 ppm had no effect on swiming speed or phytotoxic responses	Forward and Costlow, 1978, 05008745

The available data provide sufficient information to characterize methoprene as highly toxic to estuarine and marine invertebrates. No additional testing is needed.

Effects on Reptiles and Amphibians

There are no minimum data requirements for establishing the acute and/or chronic toxicity of methoprene in reptiles and amphibians but acute toxicity tests can be required depending on the use pattern of a pesticide. Acute toxicity tests on amphibians are required because methoprene is registered for direct application to aquatic habitats.

Cliburn (1973, 00010405) found that technical methoprene is moderately to slightly toxic to a variety of amphibian larvae. Very young larvae were more vulnerable to methoprene than older larvae.

In another study, exposure to 100 ppb Altosid for 24 hours slightly lowered the ability of hydrated western toads, Bufo woodhousei, to withstand high temperatures. Exposure to 200 ppb for 24 hours had no effect on toads that were dehydrated.

There is sufficient information to characterize technical methoprene as moderately toxic to amphibian larvae. No additional testing is needed.

Effects of Nontarget Soil and Surface Invertebrates

Four studies were reviewed and evaluated on the effects of methoprene on nontarget soil and surface invertebrates. The results of these studies are:

Table 1
Toxicity Studies on Nontaget Soil and Surface Invertebrates with Methoprene

Species	Formulation	Results	Citation
Convergent lady beetle (*Hippodamia convergens*)	Unknown; probably technical grade	None of the test insects survived, even at the dosage of 0.1ug/insect by topical application;	Bull, 1973, 05009665
Predaceous mite (*Amblyseius brazilli*)	Technical grade	Predaceous mites exposed through various routes to methoprene were adversely affected at concentrations of 10 to 1000 ppm.	El-Banhawy, 1977, 05009588
Parasitic wasp (*Encyrtus infelix*)	Methoprene 5E	Methoprene, applied to parasitized hemispherical scale (*Saissetia* coffeae) inhibited emergence of the parasites. Reduction in emergence of the parasites was 35% at the application rate of 2 lb a.i./100 gal	Hamlen, 1975, 05009588
Tachinid fly	Altosid 1% Granular	At the effective dosage (min. of 0.08 mg/kg/day) for controlling horn flies, tachinid flies were adversely affected.	Butler, 1973, 00010590

Based on these results, methoprene (technical or formulated product) is highly toxic to beneficial predaceous and parasitic insect species used in these studies.

Effects on Beneficial Insects

Two studies on the effects of methoprene on beneficial insects are available. The results of these studies are summarized below:

Table 2
Toxicity Studies on Beneficial Insects with Methoprene

Species	Formulation	Results	Citation
Honey bee (*Apis mellifera* *L*.)	Unknown; probably technical grade	When methoprene was topically applied to the larvae in the cells at the rate of 0.22 and 2.0 ug/larvae, about 13 to 38.5% of the emerged adults showed a number of deformities.	Hassein and Abdel-Aal, 1978, 05012139
Honey bee (*A. mellifera* *L*.)	ZR-515 technical: 70.1% a.i.	Methoprene is not toxic to the adult honey bee either by the topical application or the oral feeding.	Pedfern and Knox, 1974, 05009636

Based on these results, methoprene is shown to be non-toxic to the adult honey bee However, emerged adult bees show a number of morphological deformities when exposed to formulated methoprene at the larval stage in the cell.

Effects on Nontarget Aquatic Insects

One study was evaluated on the effects of methoprene on nontarget aquatic insects (Steelman et al., 1975, 05009393). Methoprene, 10% slow release formulation, caused a highly significant reduction in the population of Libellulidae larvae and to a lesser extent to *Tropistermus* adults. There were no adverse effects observed with the populations of *Notonecta* (adults and larvae), Corixids (adults and larvae), *Thermonectus* (adults) and Baetidae.

Effects on Algae, Fungi, and Aquatic Macrophytes

One study was available on the effects of methoprene on algae (Wurtsbaugh, 1978, 05000807). This study indicates that an aquatic solution containing 500 ppb methoprene significantly increased chlorophyll-a concentration, the ratio of heterocysts to vegetative cells, and nitrogen fixation in the blue-green algae *Aphanizomen*. Additional data are required. One adequate study on the effects of methoprene on fungi is available (Santa Luccia Mushrooms, 1979, 00011027). This study indicates that commercially grown mushrooms sustained only minor temporary injury from methoprene 5E treatments of 45 to 182 lb ai/acre, with no reduction in yield. At present, there are no data requirements for this topic.

No studies are available on the phytotoxic effects of methoprene to aquatic macrophytes. Data are required.

Effects on Terrestrial Macrophytes

One study was available on effects of methoprene on terrestrial macrophytes (Steelman, 1973, 00010957). This study indicates that rice is unaffected by single treatments of up to 4 oz. ai/acre, the highest level tested. Additional data are required.

Ecological Effects Profile: Formulated Methoprene Products

The only formulated product intended for outdoor application is the liquid Altosid SR-10. It is registered for use as a mosquito larvicide. Altosid briquettes are registered for use in small bodies of water which are not known fish habitats.

Ecological Effects Hazard Assessment

Birds

The use of methoprene as a mosquito control agent poses little hazard to avian species. The exposure rates are likely to be low, and both acute oral doses and chronic ingestions are practically non-toxic to wild waterfowl and upland gamebirds. Reproductive impairment is also highly unlikely, because the expected field residue levels (approx. 0.02 ppm) are several orders of magnitude lower than the level necessary to produce impairment (3 to 30 ppm).

Freshwater Fish

The use of methoprene as a mosquito larvicide poses minimal hazards to warm water fish. The low application rate should not produce a high enough concentration to cause acute poisonings, because methoprene is only modestly toxic to warm water fish. Methoprene's minimal potential for bioaccumulation and rapid dissipation from aquatic systems should prevent chronic exposure.

The use of the Altosid SR-10 mosquito larvicide formulation should not result in harm to cold water fish for much the same reasons. Altosid SR-10 is only slightly toxic to cold water fish. The toxicity of technical methoprene to cold water fish is unknown.

Amphibians

The use of methoprene as a mosquito larvicide poses minimal hazards to freshwater amphibians. Methoprene is somewhat more toxic to amphibians than to fish, but its low application rates and rapid dissipation characteristics should prevent aquatic concentrations from reaching levels lethal to most amphibians.

Freshwater Invertebrates

The use of methoprene as a mosquito larvicide poses some hazards to freshwater invertebrates, but major effects are unlikely. Laboratory studies found that methoprene was highly toxic to Daphnia magna, but only moderately toxic to a wide variety of other invertebrate species. The low application rates make the probability of acute poisonings remote.

Methoprene has demonstrated only a slight tendency to bioaccumulate. Its dissipation rate is rapid, with 90% of the initial concentration gone in 3 days. Complete photolysis occurs within 7 days in aqueous solutions. Therefore, aquatic invertebrates should not be affected by chronic exposures. Field studies support this conclusion. Steelman et al. (1975, 05009393), Breaud et al. (1977, 05009661), and Wan and Wilson (1975, 00010861) found that

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single or repeated applications of methoprene caused temporary reductions among a total of only 10 of 48 observed families of aquatic invertebrates. Most of the affected organisms were dipterans closely related to the target mosquitoes, or crustaceans closely related to Daphnia. Long-term effects were not observed. Therefore, local extirpations or irreversible population reductions are unlikely.

Marine and Estuarine Vertebrates and Invertebrates

Marine organisms will probably not be exposed to methoprene when it is used as a mosquito larvicide. Such usage calls for application to terrestrial or wetland areas. It would take so long for methoprene residues to pass through the terrestrial/estuarine and estuarine/marine interfaces that the dissipation and photolytic processes should be complete by the time the residues reach the marine systems.

Estuarine organisms are likely to be exposed to methoprene when it is used as a mosquito larvicide. The hazards to estuarine vertebrates are likely to be slight, however, because of methoprene's rapid dissipation and degradation rates. The acute toxicity of methoprene to marine vertebrates is unknown, but data have not been required because the toxicity of methoprene to warmwater, freshwater fish and amphibians is known to be moderate.

The hazards from methoprene mosquito-larvicide use are likely to be greater to estuarine invertebrates than to vertebrates. Even so, the effects on estuarine invertebrates should not be severe. The estimated environmental residues immediately after an application to 6 inches of water would be only 0.02 ppm, which is more than 10X lower than the 48-hr LC_{50} of the most sensitive estuarine species found, the eastern oyster larvae (48-hr LC_{50} = 0.247 ppm). The rapid dissipation and photolysis of methoprene should prevent chronic exposures.

The most likely hazard to estuarine invertebrates will occur during periods of low salinity. Low salinity conditions dramatically increase the toxicity of methoprene to larval mud-crabs (Costlow, 1975, 05010827; Costlow, 1977, 00010860; Christiansen et al., 1977, 05009394). Heavy rainfall usually creates conditions of low salinity in an estuary, at least temporarily. If larval invertebrates are exposed to methoprene during such conditions, some population reduction could result. However, the population reductions would probably be temporary and reversible. No extirpation of local species nor irreversible effects are likely.

Algae, Fungi, and Aquatic and Terrestrial Macrophytes

No estimate of the hazards posed to algae, fungi, or aquatic or terrestrial macrophytes can be completed at this time. Toxicity data are not available.

Endangered Species

The use of methoprene as a mosquito larvicide should have no adverse effects on populations of endangered birds, mammals, or fish. If methoprene were used in a critical habitat area of one of these types of organisms, its low toxicity should prevent the loss of any endangered organisms.

Methoprene might pose hazards to populations of endangered amphibians and aquatic invertebrates. At present only one aquatic invertebrate, the Socorro isopod (*Exosphaeroma thermophilus*), is listed as endangered. The Socorro isopod is confined to a single stream in Socorro County, New Mexico. Its critical habitat is, therefore, very well defined, and applications of any pesticide in the area are unlikely.

The habitats of two endangered amphibians, the Houston toad (*Bufo houstonensis*) and the pine barrens tree frog (Hyla andersonii) are not so well defined. These frogs and toads lay their eggs in puddles, ditches, and temporary ponds, which are the sort of habitats likely to be treated with mosquito larvicides. Therefore, the larvae of these amphibians could be exposed to mosquito larvicide applications. Methoprene is highly toxic (96-hr LC_{50}=0.17 ppm) to the larvae of the Fowler's toad (*Bufo woodhousei*), a species of the same genus as the Houston toad. Expected residues of methoprene would exceed the LC_{10} of methoprene to the larvae of the Fowler's toad. Therefore, methoprene could pose a risk to the Houston toad, if the pesticide were used within the toad's critical habitat. No toxicity data were available for members of the genus *Hyla*, but the high toxicity of methoprene to *Bufo woodhousei* raises concerns about the possible effects on the pine barrens tree frog.

At the present time, a complete assessment of the hazards of methoprene to these endangered species cannot be made due to insuffficent information. A complete hazard assessment for endangered species will be done pending receipt of further information and consultation with the Office of Endangered Species.

Chapter IX

Efficacy

Efficacy Profile

The review of methoprene data and the identification of data gaps are limited to efficancy data only as it relates to public health applications. In all other instances the Agency has provided for the waiver of efficancy data submission as a part of the registration process (44 FR 27932, May 11, 1979). Efficacy data will be required for products intended for use either in premises or in the environment to control pests of public health significance.

Methoprene is an insect growth regulator and is used as a larvicide to control mosquito larvae. Since the control of mosquitoes is considered to be of public health significance, efficacy data are required to support the registration of methoprene products.

In reviewing the site terminology from currently registered labels and available efficacy data for methoprene, there are a number of sites for which there are not adequate efficacy data to support label claims. These sites are: cesspools, septic tanks, roadside ditches, ornamental ponds and fountains, estuarine areas, drainage areas and dredging spoil sites. However, except for three sites, most of these sites can be considered equivalent to other sites for which adequate data do exist. There are insufficient data to support claims of efficacy in controlling mosquito larvae in cesspools, septic tanks, and waste treatment and settling ponds. Requested data are listed in Table C of Chapter III.

Factors Influencing Efficacy

There are a number of factors which directly effect the efficacy of methoprene. These factors include, but may not be limited to, the following: formulation type, exposure to sunlight, temperature, carrier, water flow or fluctuations, the amount of organic debris, application timing, and type of pest (one genus of mosquitoes versus another). Several studies investigating one or more of the above factors influencing the efficacy of methoprene are available and are discussed below. While all available literature was reviewed, not all studies are cited in this discussion.

1. Formulation Type

Mulla (1976, 05020794) found methoprene formulated in briquettes provided much greater residual and, therefore, greater control than sand granule formulations. Hazelrigg (1979, 05020795) obtained the greatest residual effect with methoprene formulated in charcoal discs or "pies." This work led to the registration of California Special Local Need No. 180183 for the use of such discs. Spencer, Olson, and McNeill (1979, 05020219) found the slow release formulation (SR-10F) to have a greater residual effect than the emulsifiable concentrate formulation.

2. Exposure to Sunlight

Spencer *et al*. (1979, 05020219) evaluated a number of factors that effect the efficacy of methoprene. They observed rapid degradation of methoprene after prolonged exposure to sunlight. Mosquito control lasted longer in shaded source habitats.

3. Water Temperature

Mulla (1975, 05009928) investigated the effect of water temperature on the efficacy of methoprene. In this study, it was noted that high water temperatures caused methoprene to degrade. Mulla postulates that the high temperature affected the breakdown of methoprene by causing a rapid release from the capsules or a fast degradation of the compound in water.

4. Carrier or Delivery Medium

Rogers et al. (1976, 05008608) obtained excellent control of Aedes taeniorhynchus in sources located under dense mangrove and pickleweed canopies by using sand as a carrier. The customary water emulsion merely settled on the vegetation, did not penetrate the canopy and by not reaching the water source, did not control the mosquitoes.

Wagestaff (1974, 05015768) also found sand to be a more efficient carrier than water in instances involving dense canopies. None of the material is lost on the vegetation as is the case with liquid formulations. In another study, Wagestaff and Minson (1974, 05015768) observed pools subject to reflooding (open water system sources) and noted that the methoprene treatments were ineffective in controlling mosquitoes. In adjacent closed system pools, not subject to reflooding, methoprene treatments effectively controlled mosquitoes.

5. Organic Debris in Source

Stewart (1977, 05009592) observed a drastic decrease in control of mosquitoes when excessive amounts of organic matter contaiminated the water. In this particular study, the debris seemed to inhibit the release of methoprene from the briquettes. The methoprene, therefore, could not rise to the surface of the water where it could contact the mosquito larvae.

6. Life Stage

Vorgetts (1976, 05010646) noted the greatest control was obtained when third and fourth instar larvae were treated versus first or second instar larvae and pupae. This factor is particularly important when methoprene is sprayed. Briquettes provide a greater residual effect and increase the effective length of treatment time. Vorgetts stated that timing was of critical importance expecially if emulsions are being considered for use.

7. Type of Pest (Genera of mosquitoes)

A number of studies noted less effectiveness in controlling Culex spp. than Aedes spp. mosquitoes using identical techniques. At the low registered rates of application, Culex mosquitoes do not seem to be as severely affected by methoprene as are other genera (Schaefer, 1972, 00011005; Wagstaff, 1974, 05015768; Sjogren, 1976, 00011065).

Use Sites

Only use sites associated with the protection of public health are addressed in the efficacy review. The sites reviewed for methoprene were considered because of their relationship as mosquito breeding habitats. These sites are: pastures; rice; catch basins; cesspools; ditches; drainage areas; dredging spoil sites; estuarine areas; intermittently flooded areas (including abandoned swimming pools, flooded crypts, natural and manmade depressions, transformer vaults); marshes and swamps; ornamental fountains and ponds; roadside ditches; salt and tidal marshes; septic tanks; storm drains; waste treatment and settling ponds; woodland meadows and woodland pools.

Target Pests

The EPA Index lists the larvae of several genera of mosquitoes as the target pests. The pests are: the floodwater complex (commonly accepted to mean certain species of *Aedes* and *Psorophora*) and the genera *Anopheles*, *Culex*, and *Culiseta*. Current labels do not combine all of these pest groups with each site of application. For example, only the floodwater complex is registered to be controlled in salt and tidal marshes, whereas roadside ditches are associated with the floodwater complex as well as species of *Anopheles*, *Culex*, and *Culiseta*.

Chapter X

Case Bibliography

Guide to Use of This Bibliography

1. CONTENT OF BIBLIOGRAPHY. This bibliography contains citations of all the studies reviewed by EPA in arriving at the positions and conclusions stated elsewhere in the Standard. The bibliography is divided into 3 sections: (1) citations that contributed information useful to the review of the chemical and considered to be part of the data base supporting registrations under the Standard, (2) citations examined and judged to be inappropriate for use in developing the Standard, and (3) standard reference material. Primary sources for studies in this bibliography have been the body of data submitted to EPA and its predecessor agencies in support of past regulatory decisions, and the published technical literature.

2. UNITS OF ENTRY. The unit of entry in this bibliography is called a "study." In the case of unpublished materials submitted to the Agency, the Agency has sought to identify documents at a level parallel to the published article from within the typically larger volumes in which they were submitted. The resulting "studies" generally have a district title (or at least a single subject), can stand alone for purpose of review, and can be described with a conventional bibliographic citation. The Agency also has attempted to unite basic documents and commentaries upon them, treating them as a single study.

3. IDENTIFICATION OF ENTRIES. The entries in this bibliography are sorted by author, date of the document, and title. Each entry bears, to the left of the citation proper, an eight-digit numeric identifier. This number is unique to the citations, and should be used at any time specific reference is required. This number is called in the "Master Record Identifier," or "MRID." It is not related to the six-digit "Accession Number" which has been used to identify volumes of submitted data; see paragraph 4(d)(4) below for a further explanation. In a few cases, entries added to the bibligraphy late in the review may be preceded by a nine-character temporary identifier. This is also to be used whenever specific reference is needed.

4. FORM OF THE ENTRY. In addition to the Master Record Identifier (MRID), each entry consists of a bibliographic citation containing standard elements followed, in the case of materials submitted to EPA, by a description of the earliest known submission. The bibliographic conventions used reflect the standards of the American National Standards Institute (ANSI), expanded to provide for certain special needs. Some explanatory notes of specific elements follow:

   a. Author. Whenever the Agency could confidently identify one, the Agency has chosen to show a personal author. When no individual was identified, the Agency has shown an identifiable laboratory or testing facility as author. As a last resort, the Agency has shown the first known submitter as author.

b. Document Date. When the data appears as four digits with no question marks, the Agency took it directly from the document. When a four- digit date is followed by a question mark, the bibliographer deduced the date from evidence in the document. When the date appears as (19??), the Agency was unable to determine or estimate the date of the document.

c. Title. This is the third element in the citation. In some cases it has been necessary for Agency bibliographers to create or enhance a document title. Any such editorial insertions are contained between square brackets.

d. Trailing Parentheses. For studies submitted to the Agency in the past, the trailing parentheses include (in addition to any self-explanatory text) the following elements describing the earliest known submission:

(1) Submission Date. Immediately following the word "received" appears the date of the earliest known submission.

(2) Adminstrative Number. The next element, immediately following the word "under," is the registration number, experimental permit number, petition number, or other administrative number associated with the earliest known submission.

(3) Submitter. The third element is the submitter, following the phrase "submitted." When authorship is defaulted to the submitter, this element is omitted.

(4) Volume Identification. The final element in the trailing parenthesis identifies the EPA accession number of the volume in which the original submission of the study appears. The six-digit accession number follows the symbol "CDL," standing for "Company Data Library." This accession number is in turn followed by an alphabetic suffix which shows the relative position of the study within the volume. For example, within accession number 123456, the first study would be 123456-A; the second, 123456-B; the 26th, 1232456-Z; and the 27th, 123456-AA.

OFFICE OF PESTICIDE PROGRAMS
REGISTRATION STANDARD BIBLIOGRAPHY
Citations Evaluated and Considered to be Part of the Data Base Supporting Registration Under the Standard

000001234 Anon. (19??) Method Tryout for Altosid in Meat and Milk performed on Method No. 067 "The Analysis of Altosid in Cow's Milk."

00010443 Aschooley (sic), D.; Bergot, B.J. (19??) 10-3H Altosid Dissipation Rates in Pond Water and Sewage. Includes undated method. (Unpublished study received Dec. 5, 1972 under 20954-EX-1; submitted by Zoecon Corp., Palo Alto, Calif.; CDL:127777-AK)

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